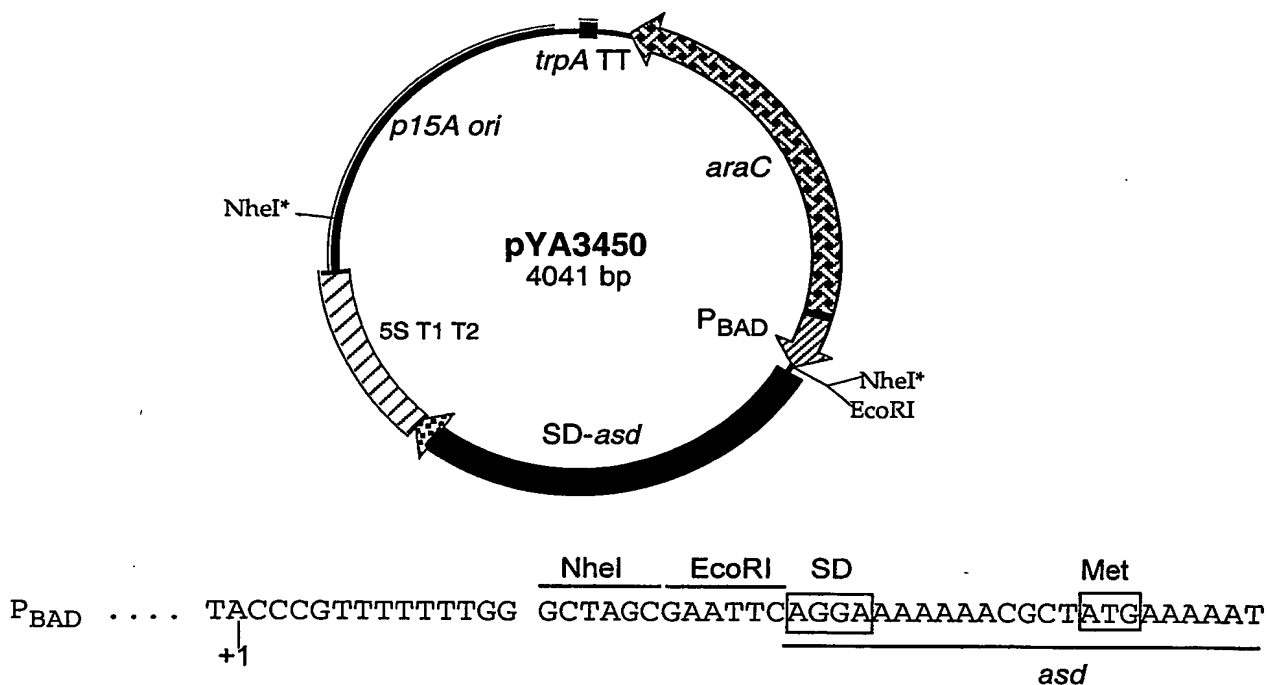


FIGURE 1. pYA3450



pYA3450 is an *araC* *P<sub>BAD</sub>* *asd* vector derived from pYA247. Around 35 bp (the number was calculated based on the map of pYA247 from MEGAN) unnecessary sequence between *P<sub>BAD</sub>* and *asd* was deleted by replacing the original *asd* gene with a 1,100 bp *EcoRI*-*HindIII* PCR product derived from the *asd* gene of pYA292. The PCR product contains the *asd* gene from 300 to 1420 bp including the SD sequence. The pYA247 was cut with *EcoRI* and *HindIII* and the 2.9 kb fragment was isolated and ligated with the 1.1 kb PCR product of *asd*.

FIGURE 2A. pYA3530

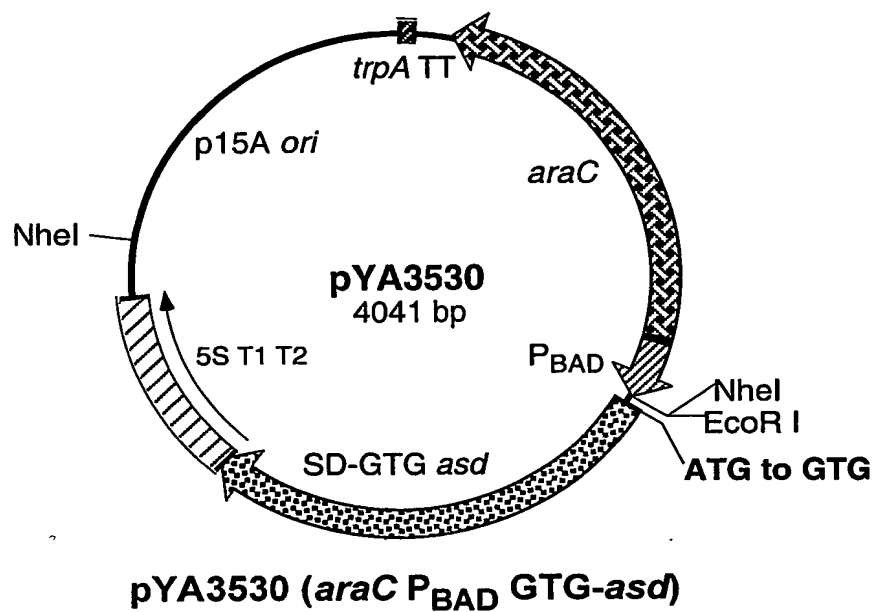
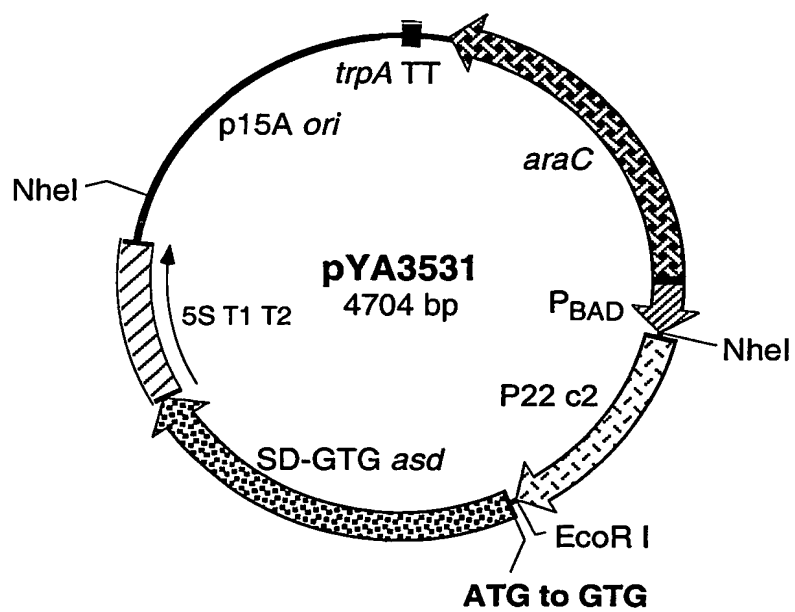


FIGURE 2B. pYA3531



pYA3531 (*araC* *P<sub>BAD</sub>* *P22 c2* GTG-*asd*)

**FIGURE 3. Growth of  $\chi 8645 \Delta P_{murA7}::araC P_{BAD} murA$  in 1% Rodent Chow, 1% Chicken Feed and 1% Chicken Breast Meat Broth +/- 0.5% Arabinose**

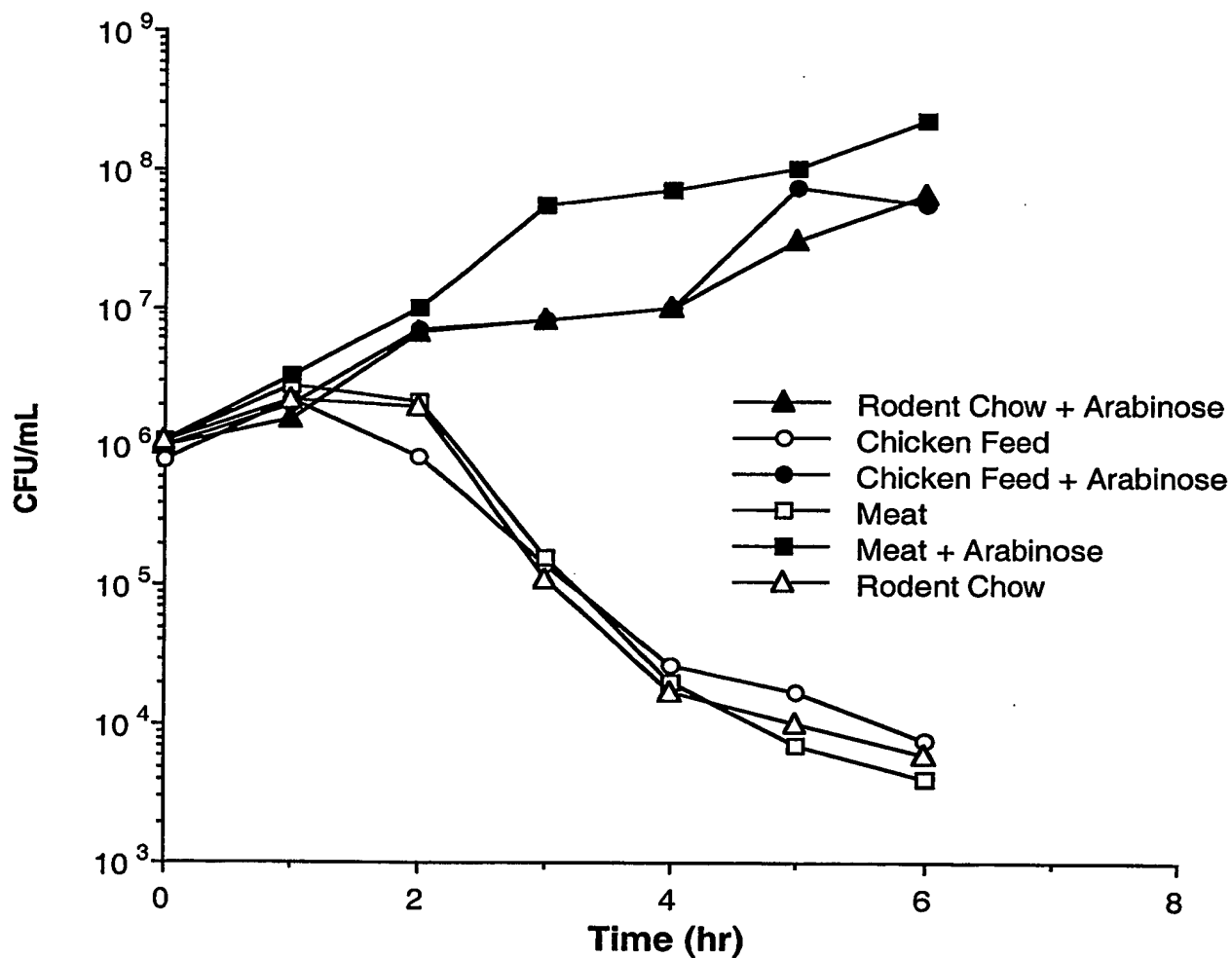
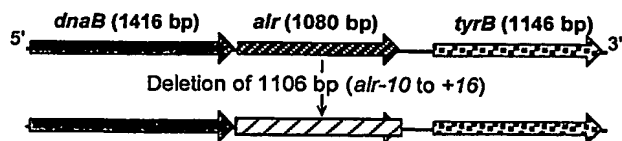
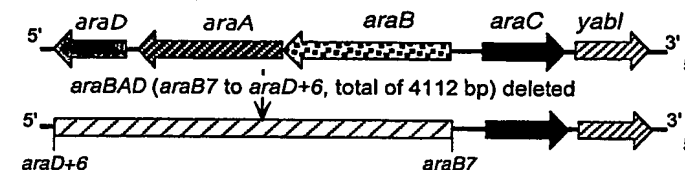
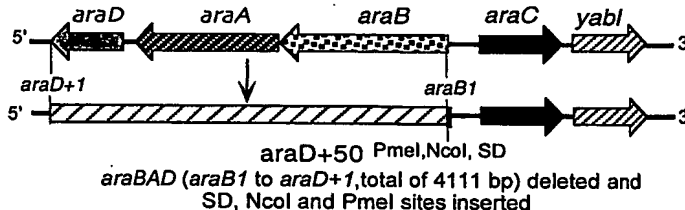
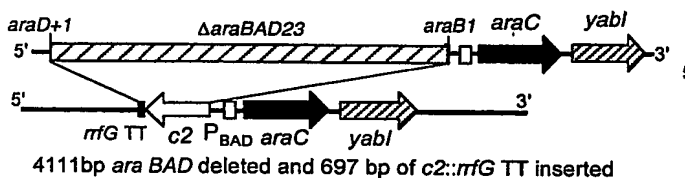
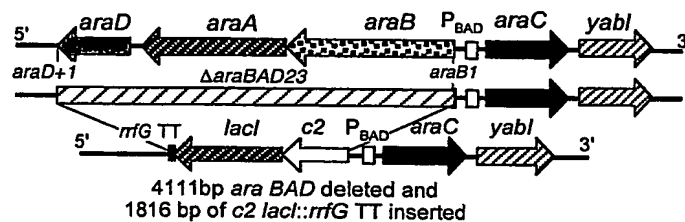
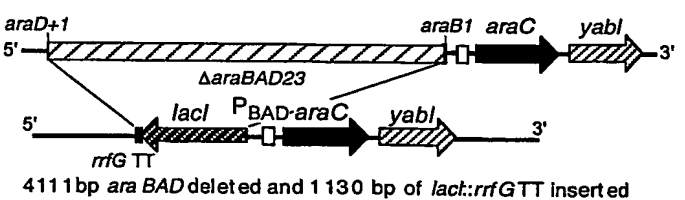
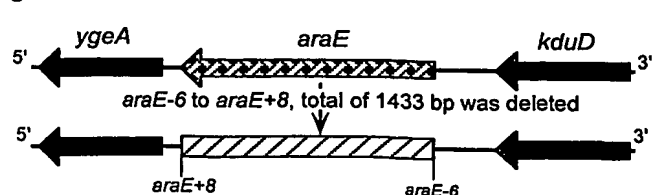
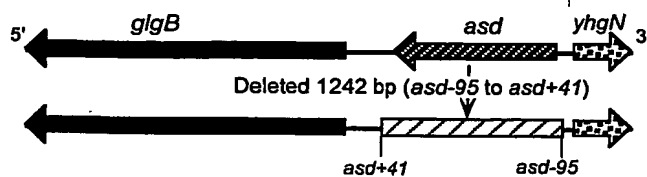
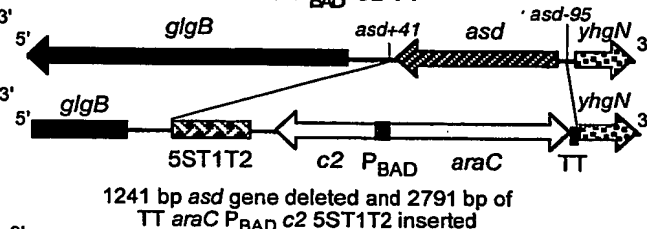
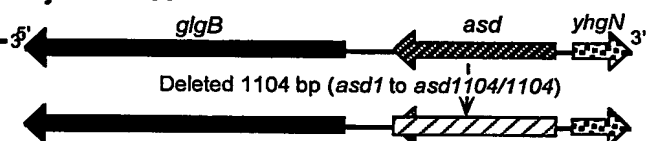
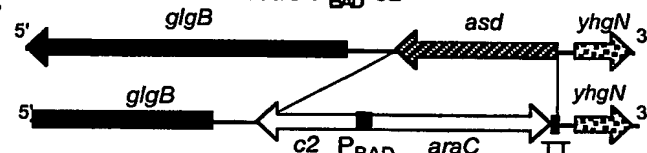
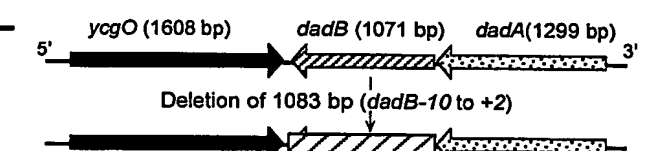
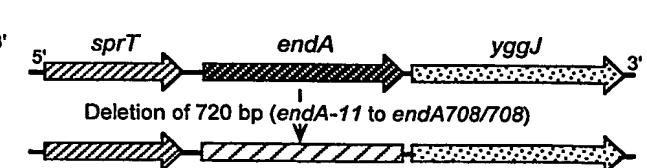
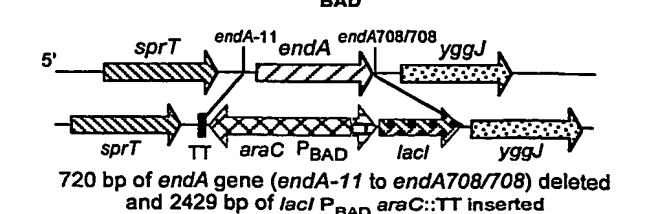
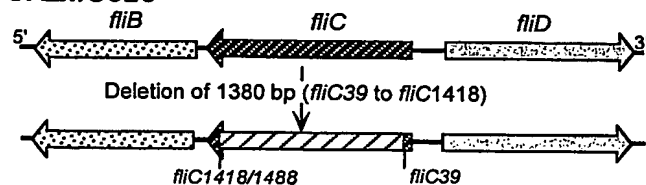
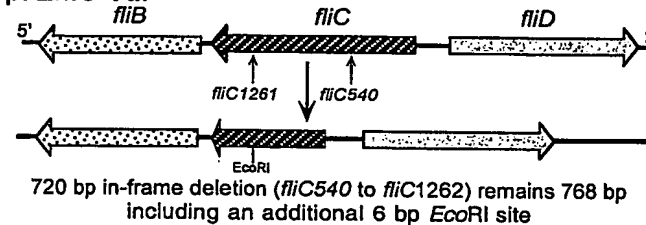
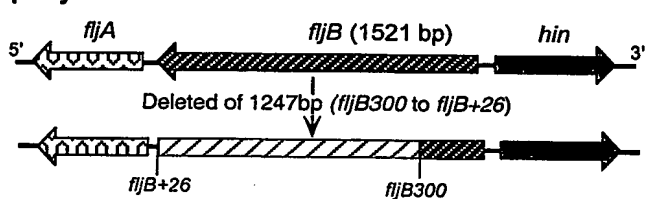
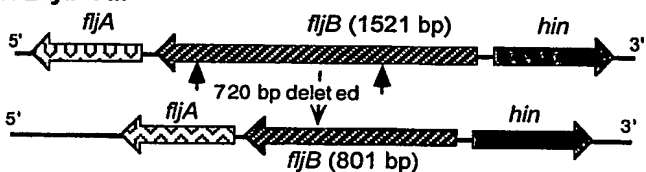
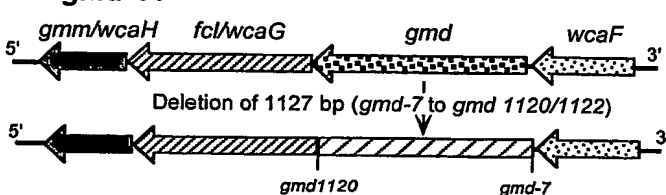
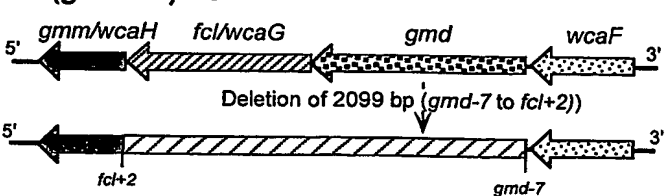
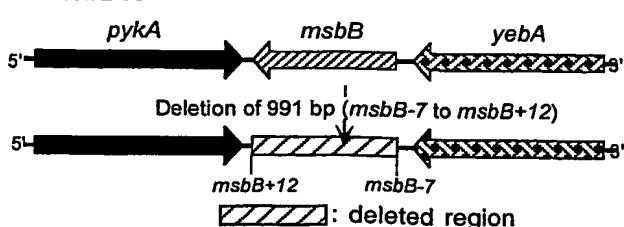
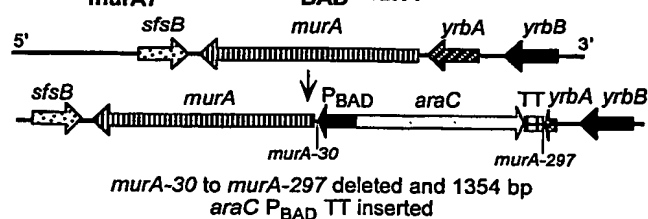
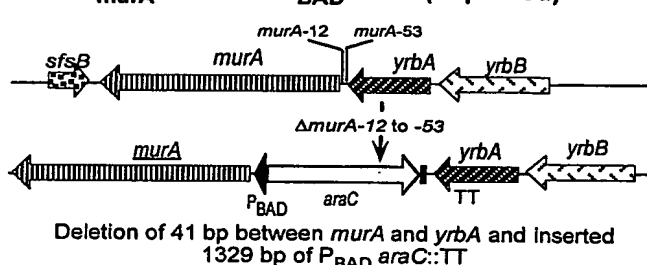
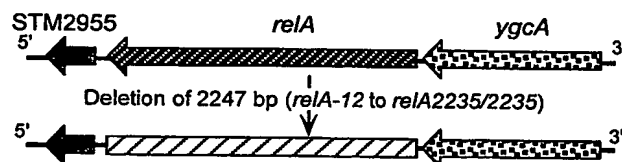
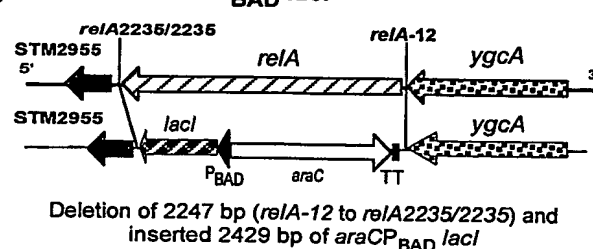
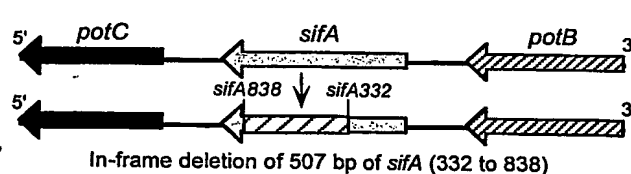
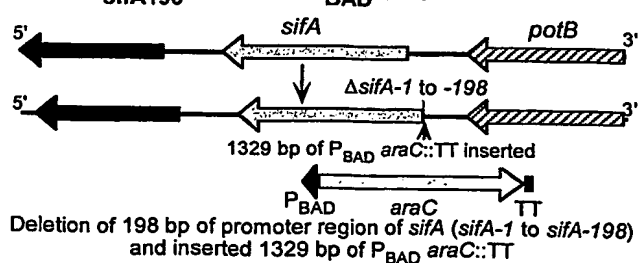


FIGURE 4. Structure of deletion and deletion-insertion mutations

a.  $\Delta alr-3$ b.  $\Delta araBAD1923$ c.  $\Delta araBAD23$ d.  $\Delta araBAD23$  c2::TTe.  $\Delta araBAD23$  c2 *lacI*::TTf.  $\Delta araBAD23$  *lacI*::TTg.  $\Delta araE25$ h.  $\Delta asdA16$ i.  $\Delta asdA19$ ::TT *araC* P<sub>BAD</sub> c2 TTj.  $\Delta asdA33$ k.  $\Delta asdA183$ ::TT *araC* P<sub>BAD</sub> c2l.  $\Delta dadB4$ m.  $\Delta endA2311$ n.  $\Delta endA23$ ::TT *araC* P<sub>BAD</sub> *lacI*

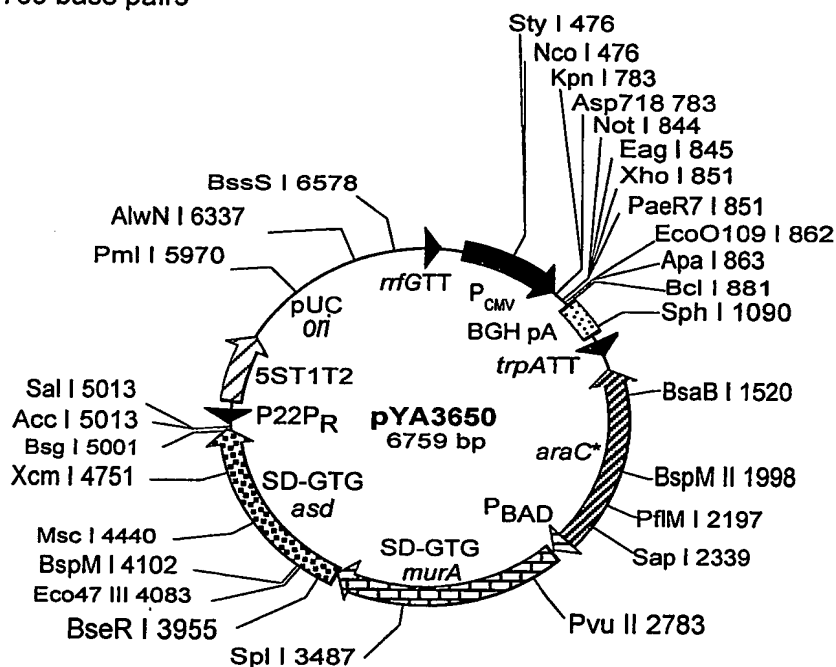
▨: deleted region

FIGURE 4. continued

o.  $\Delta fliC825$ p.  $\Delta fliC$ -Varq.  $\Delta fljB217$ r.  $\Delta fljB$ -Vars.  $\Delta gmd-11$ t.  $\Delta(gmd-fcl)$ -26u.  $\Delta msbB48$ v.  $\Delta P_{murA7}::TT$  *araC*  $P_{BAD}$  *murA*w.  $\Delta P_{murA}::TT$  *araC*  $P_{BAD}$  *murA* (improved)x.  $\Delta relA1123$ y.  $\Delta relA::TT$  *araC*  $P_{BAD}$  *lacI*z.  $\Delta sifA26$ aa.  $\Delta P_{sifA196}::TT$  *araC*  $P_{BAD}$  *sifA*

# FIGURE 5A. DNA vaccine vector pYA3650

6,759 base pairs



DNA vaccine vector pYA3650 designed to express eukaryotic DNA from human cytomegalovirus immediate early gene promoter.

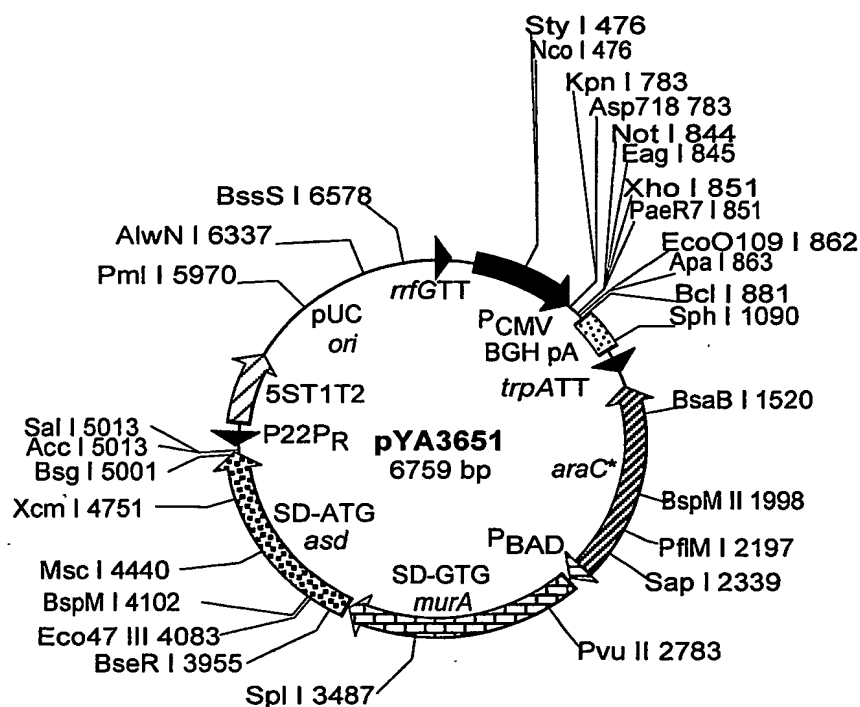
pYA3650 contains the immediate early gene promoter / enhancer from CMV, the Bovine growth hormone (BGH) gene polyadenylation signal, transcriptional termination sequence to enhance mRNA stability, a regulatable activator-promoter complex controlling the in vitro/in vivo expression of two genetically modified genes specifying enzymes necessary for synthesis of the rigid layer of the bacterial cell wall, a regulated synthesis of antisense RNA to completely turn off in vivo translation of mRNA encoded by the two genes whose products are necessary for synthesis of the rigid layer of the bacterial cell wall, and a replicon necessary for replication in bacteria but not in eukaryotic cells.

The exact locations are: *rrfGTT* 36-100, *trpATT* 1243-1271, SD-GTG *murA* 2595/3868, 5ST1T2 5178/5620, P<sub>CMV</sub> 202-789, *araC* 2497-1377, SD-GTG *asd*, 3889/4995, pUC *ori* 6081/6754, BGH pA 888/1118, P<sub>BAD</sub> 2498-2582 and P22P<sub>R</sub> 5106-5027.

The map shows the location of sites for enzymes that cleave the molecule once.

**FIGURE 5B. DNA vaccine vector pYA3651**

6,759 base pairs

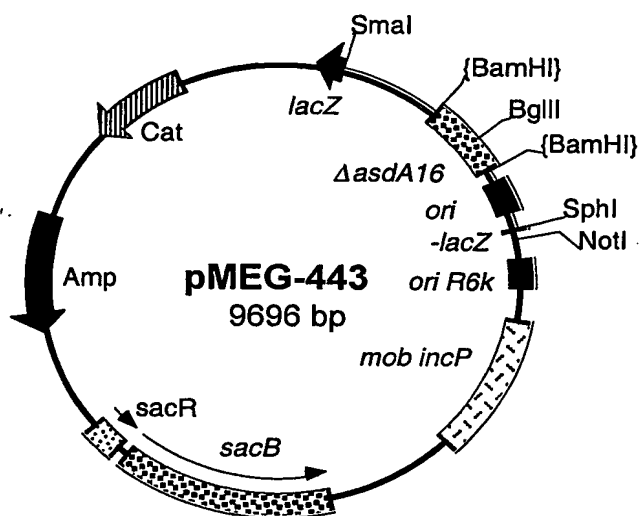
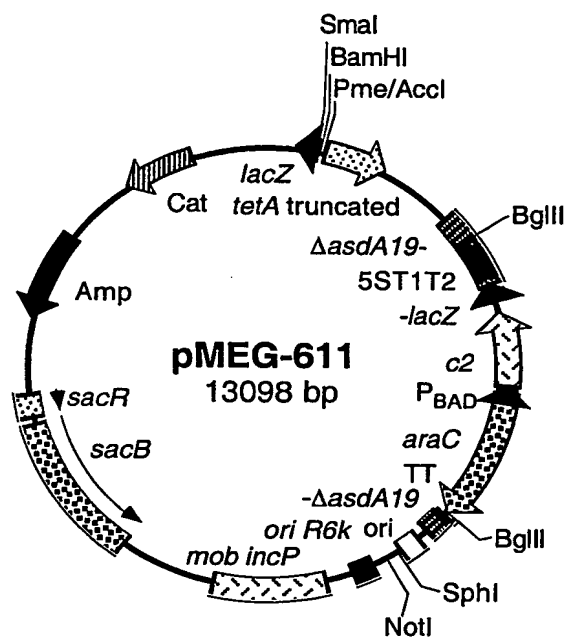
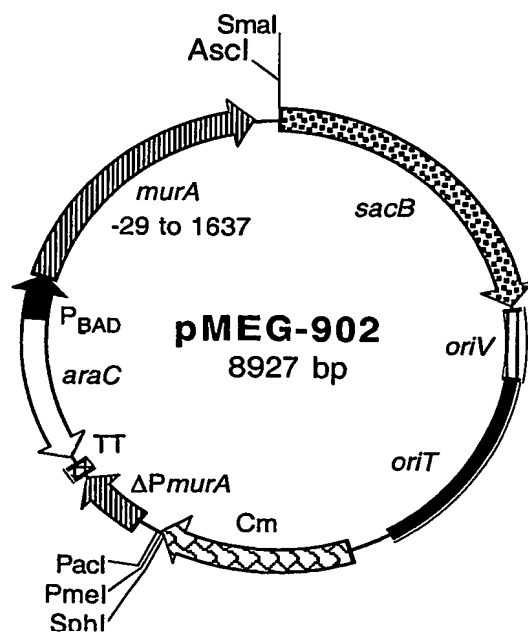


DNA vaccine vector pYA3651 possesses SD-ATG-*asd* designed to increase translational level of Asd mRNA compared to pYA3650. The exact locations are: *rrfGTT* 36-100, *trpATT* 1243-1271, SD-GTG *murA* 2595/3868, 5ST1T2 5178/5620, P<sub>CMV</sub> 202-789, *araC\** 2497-1377, SD-ATG *asd*, 3889/4995, pUC *ori* 6081/6754, BGH pA 888/1118, P<sub>BAD</sub> 2498-2582 and P22P<sub>R</sub> 5106-5027.

The map shows the location of sites for enzymes that cleave the molecule once.



FIGURE 6. Suicide Vectors

A.  $\Delta asdA16$ B.  $\Delta asdA19::TT araC P_{BAD} c2$ C.  $\Delta P_{mur7}::TT araC P_{BAD} murA$ 

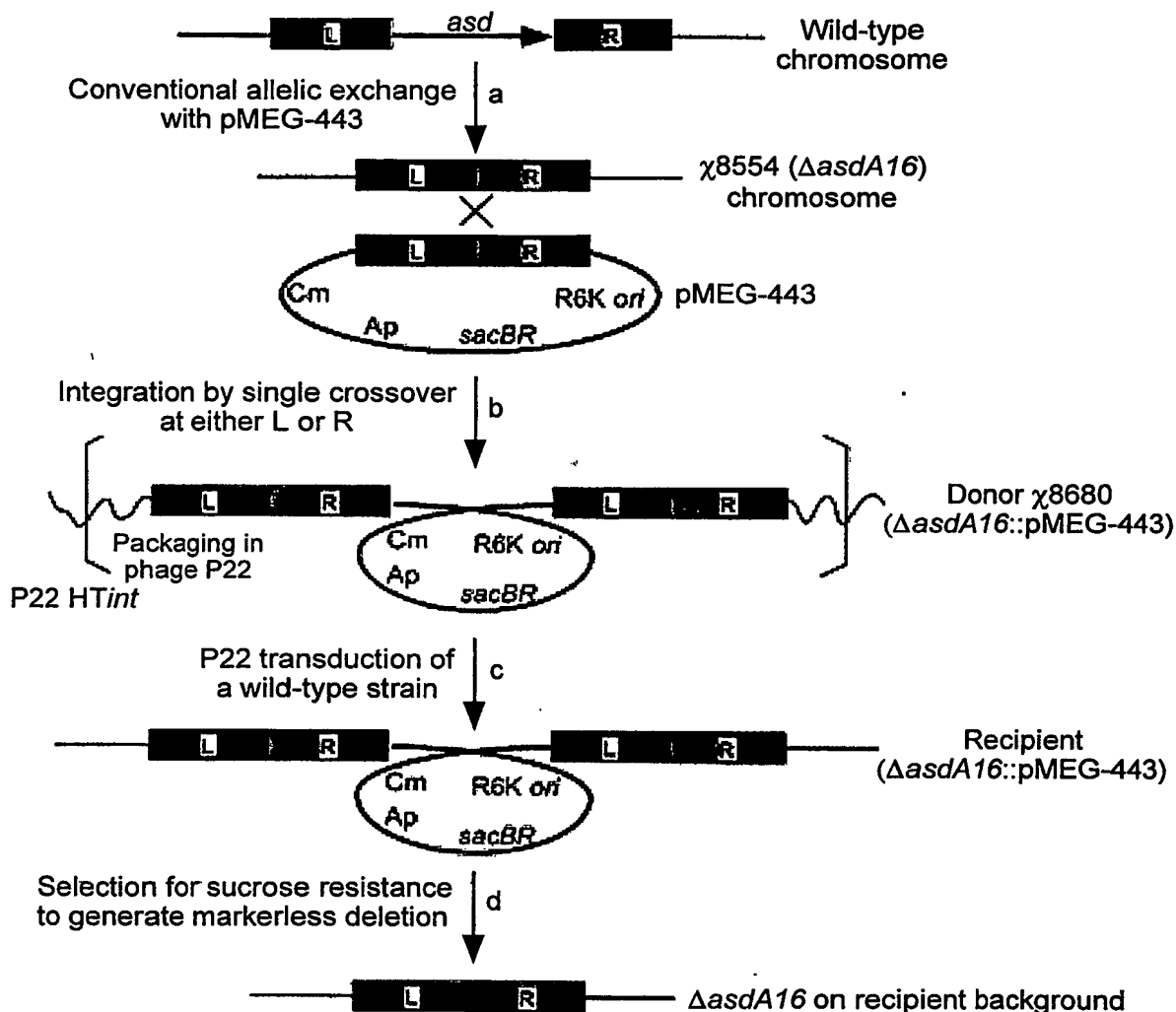
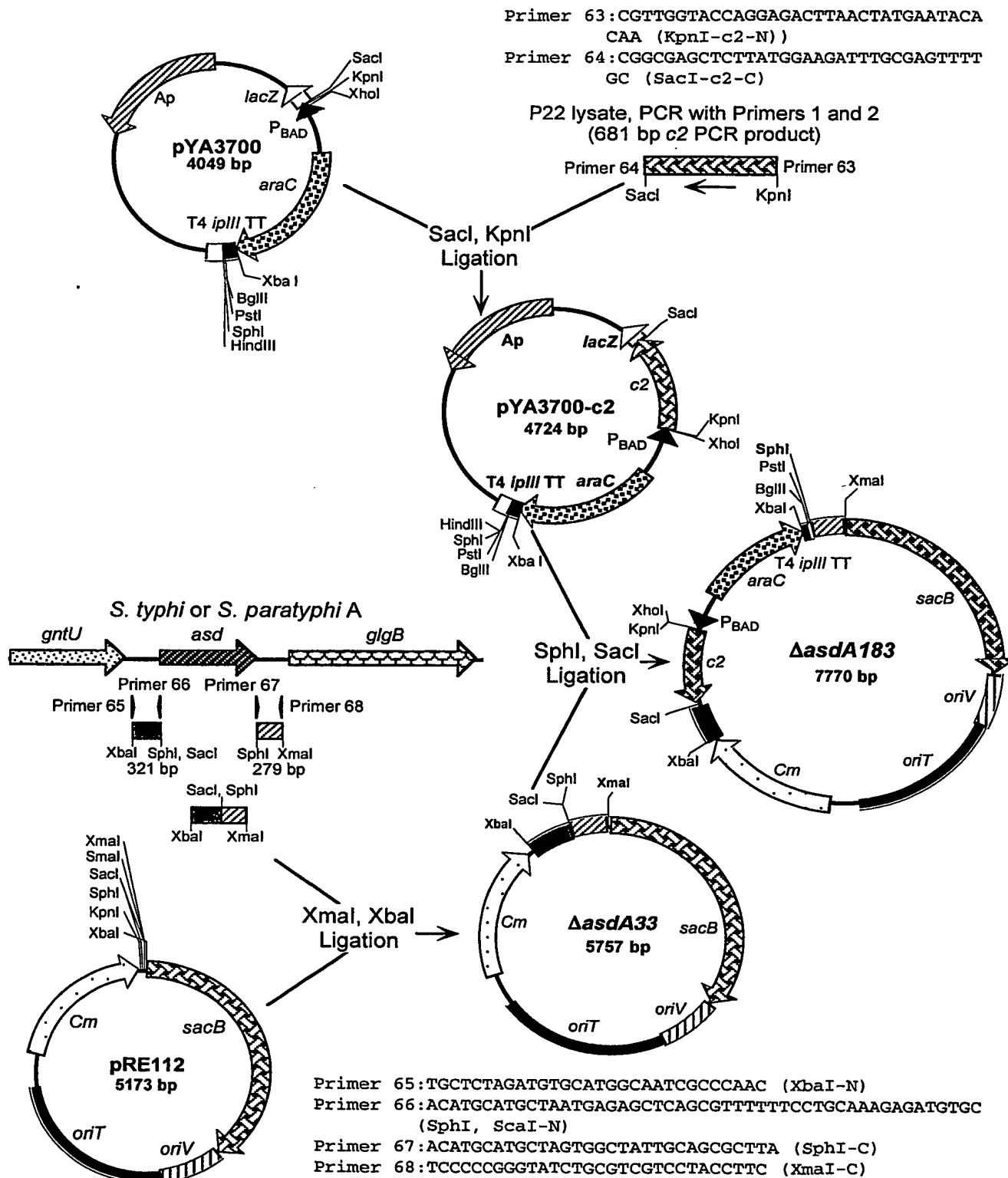
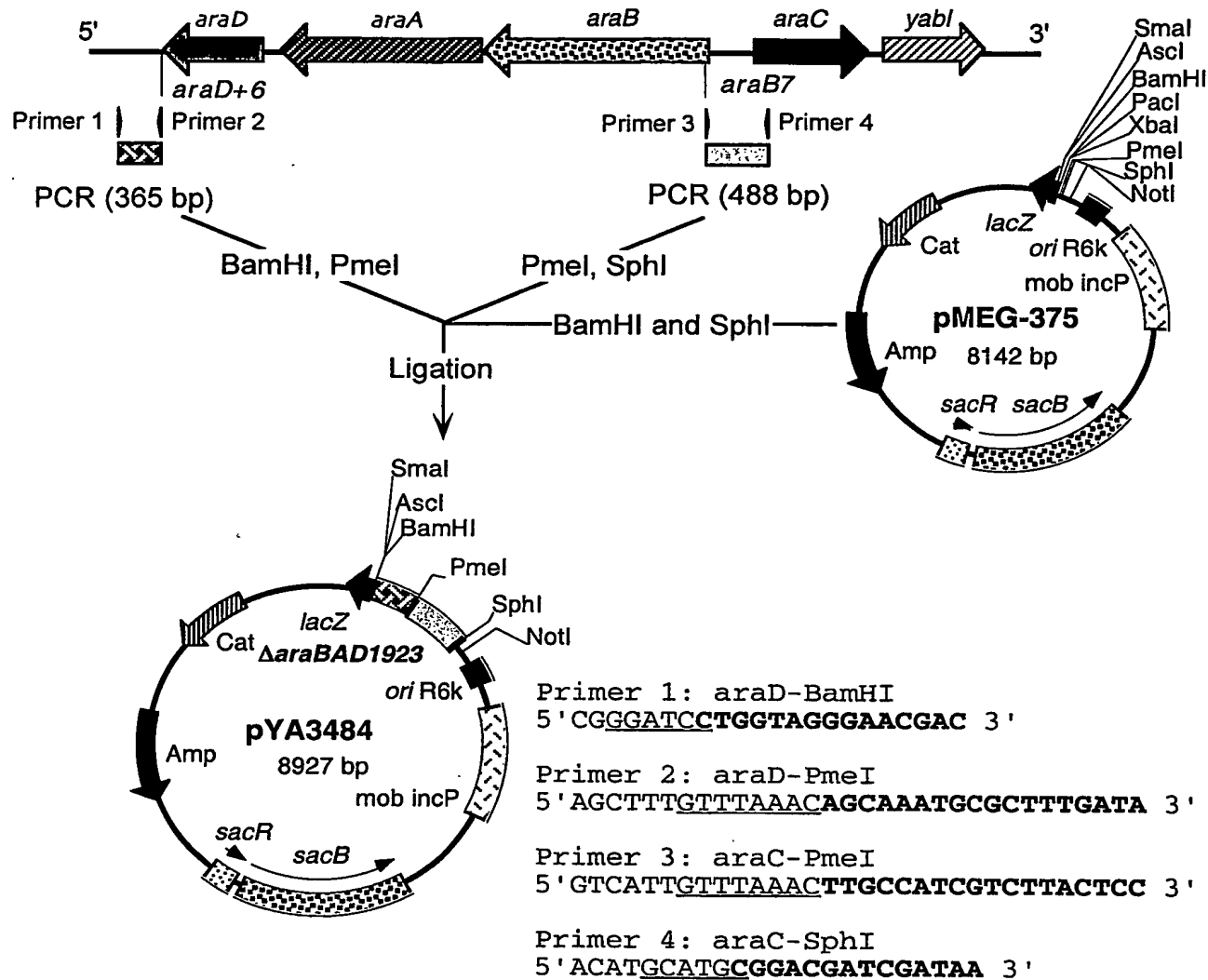
**FIGURE 7. Transductional Method of Moving Unmarked Mutations**

Illustration of overall processes for transfer of the  $\Delta$ *asdA16* mutation. Black boxes and gray boxes represent cloned 5' (left [L]) and 3' (right [R]) flanking regions, respectively, of the *asd* gene. (Step a) Using the recombinant suicide plasmid pMEG-443, a  $\Delta$ *asdA16* mutant was generated by the routine allelic-exchange method. (Step b) Plasmid pMEG-443 was integrated into the chromosome of the  $\chi$ 8554 ( $\Delta$ *asdA16*) strain by single-crossover insertion. (Step c) Phage P22HTint was propagated on the donor strain  $\chi$ 8680 ( $\Delta$ *asdA16*::pMEG-443). The  $\Delta$ *asdA16*::pMEG-443 complex was transduced to a wild-type recipient strain, and transductants were selected based on the plasmid-encoded antibiotic resistance markers. (Step d) Excision of the plasmid by homologous recombination between duplicated regions was selected for by using the plasmid-carried *sacB* counterselection system to generate the unmarked deletion mutation.

**FIGURE 8. Construction of suicide vectors  $\Delta asdA33$  and  $\Delta asdA183::TT araC P_{BAD} c2$  for use in *S. typhi* and *S. paratyphi* A**



**FIGURE 9. Construction of suicide vector for  $\Delta$ araBAD1923**



**In *Salmonella* chromosome:**

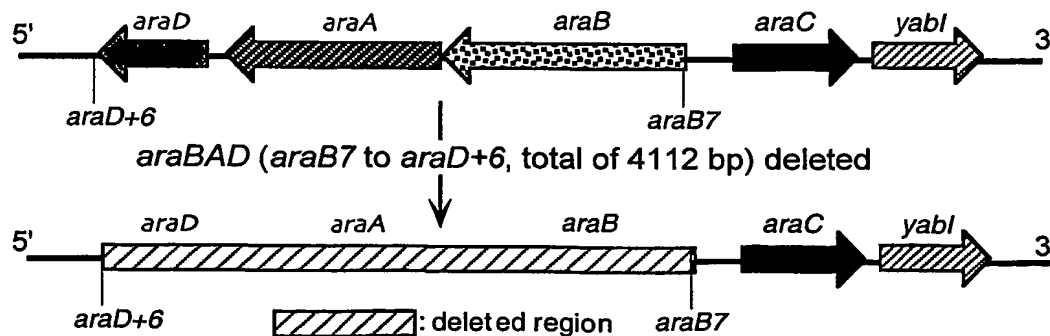
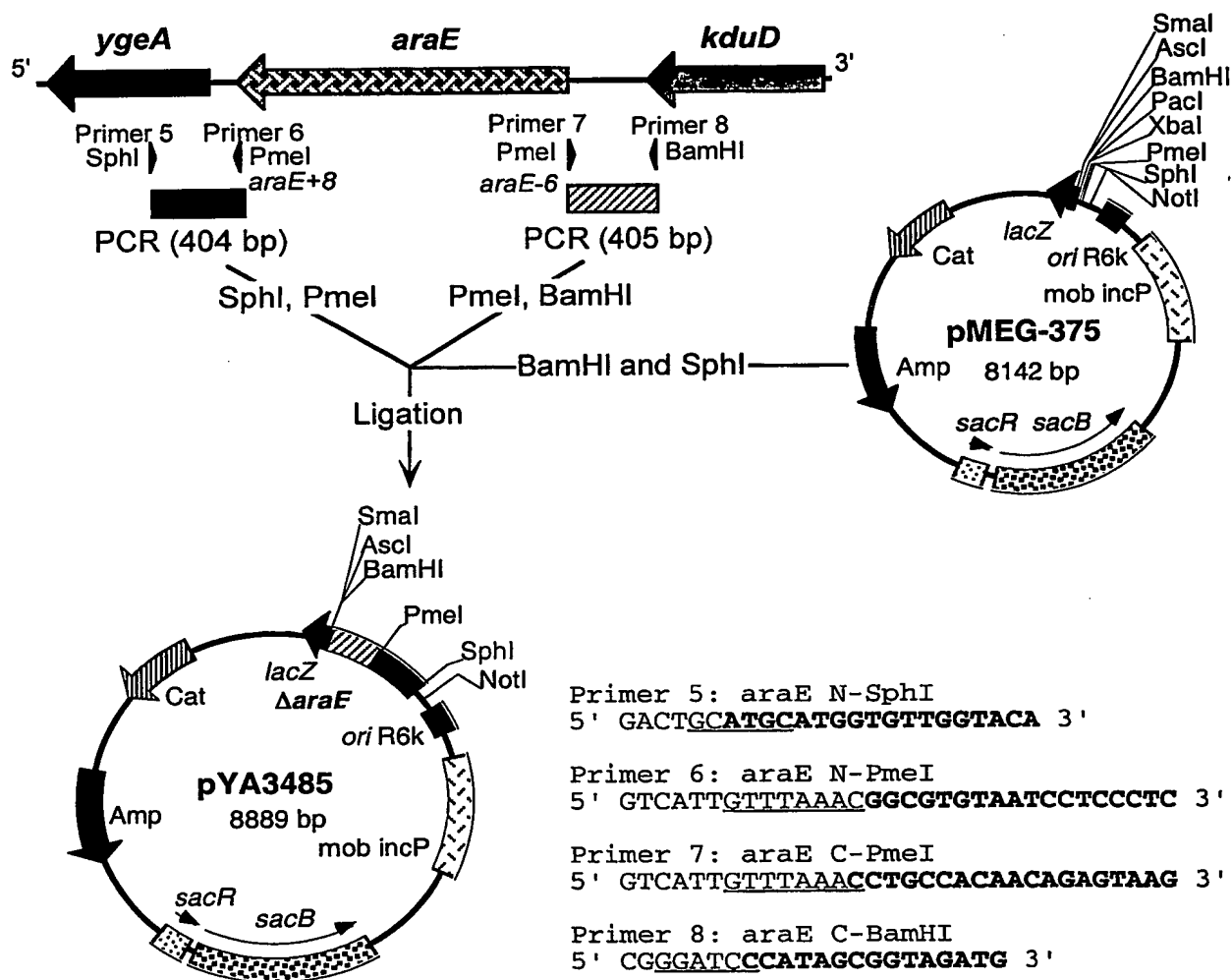
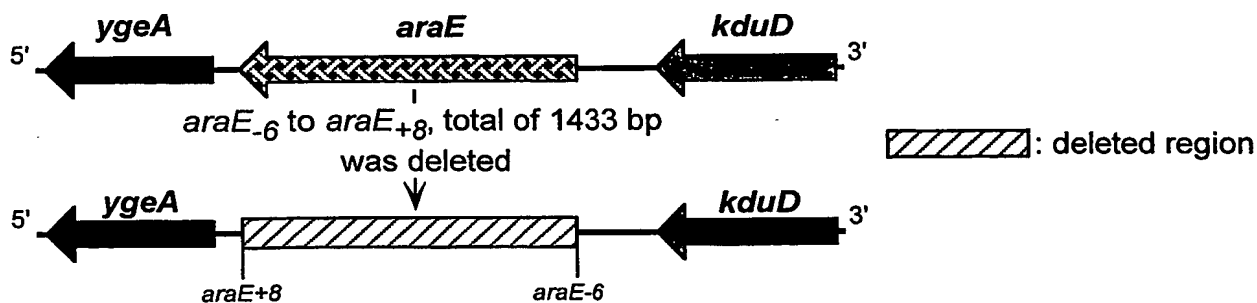


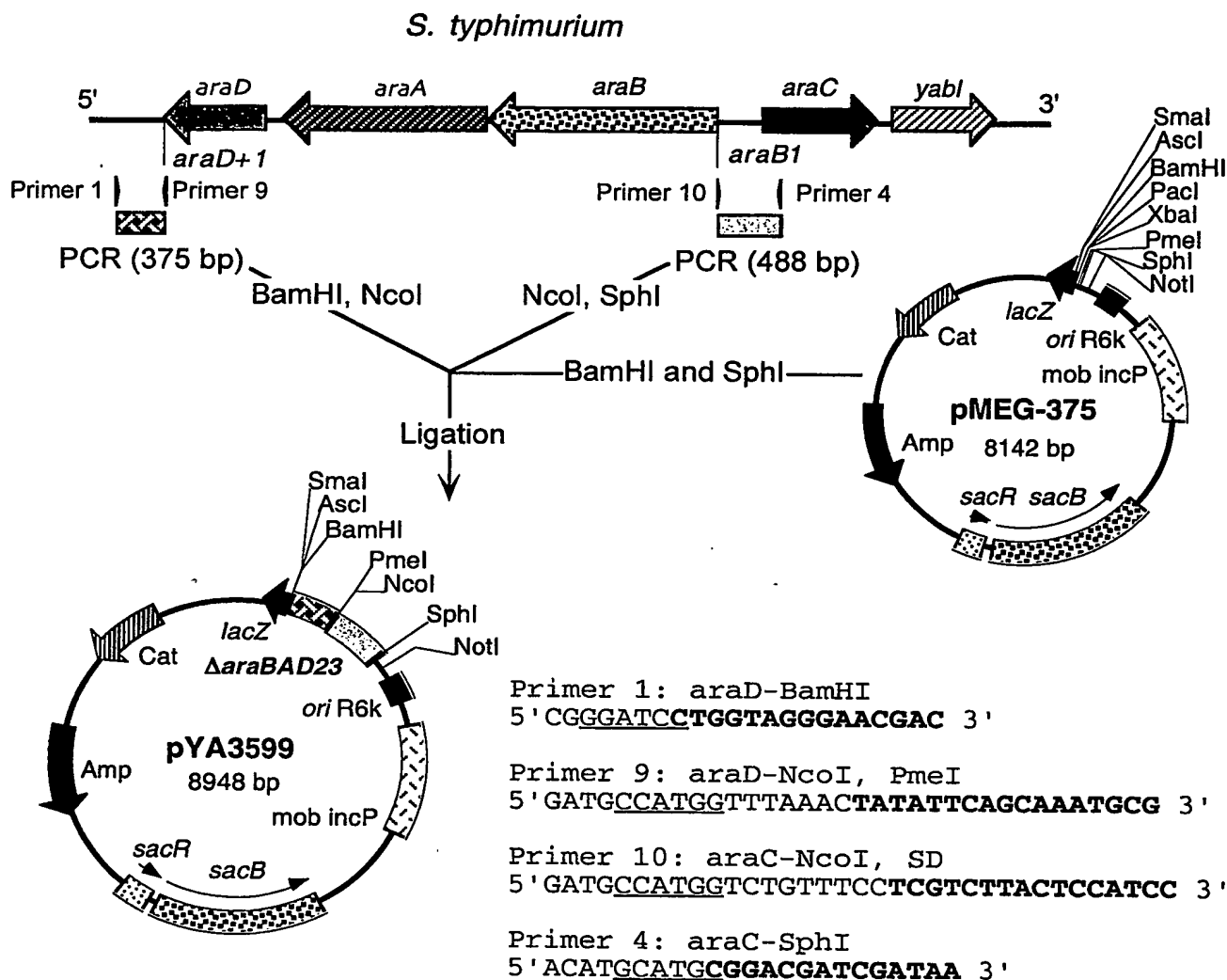
FIGURE 10. Construction of suicide vector for  $\Delta$ araE25In *Salmonella* chromosome:

ygeA: Function unknown

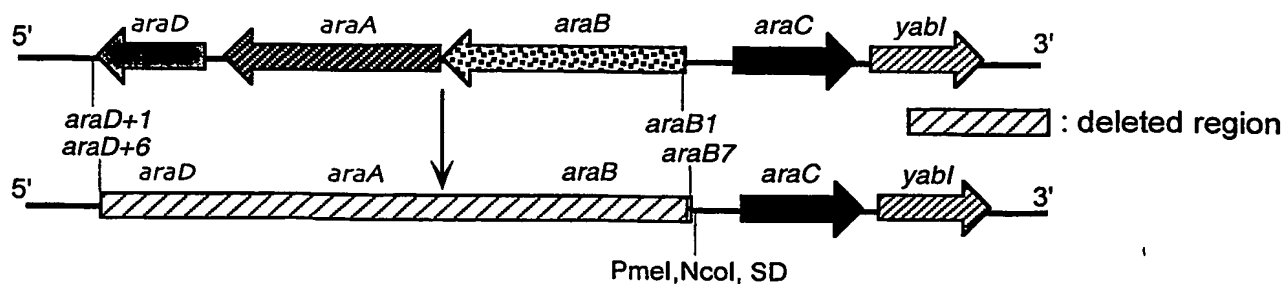
araE: Low-affinity L-arabinose transport; L-arabinose proton symport

kduD: 2-deoxy-D-gluconate 3-dehydrogenase

**FIGURE 11. Construction of suicide vector for  $\Delta$ araBAD23**



**In *Salmonella* chromosome:**



**FIGURE 12. Construction of suicide vectors for  $\Delta araBAD23$   $c2$   $lacI::rrfG$  TT,  $\Delta araBAD23$   $c2::rrfG$  TT and  $\Delta araBAD23$   $lacI::rrfG$  TT**

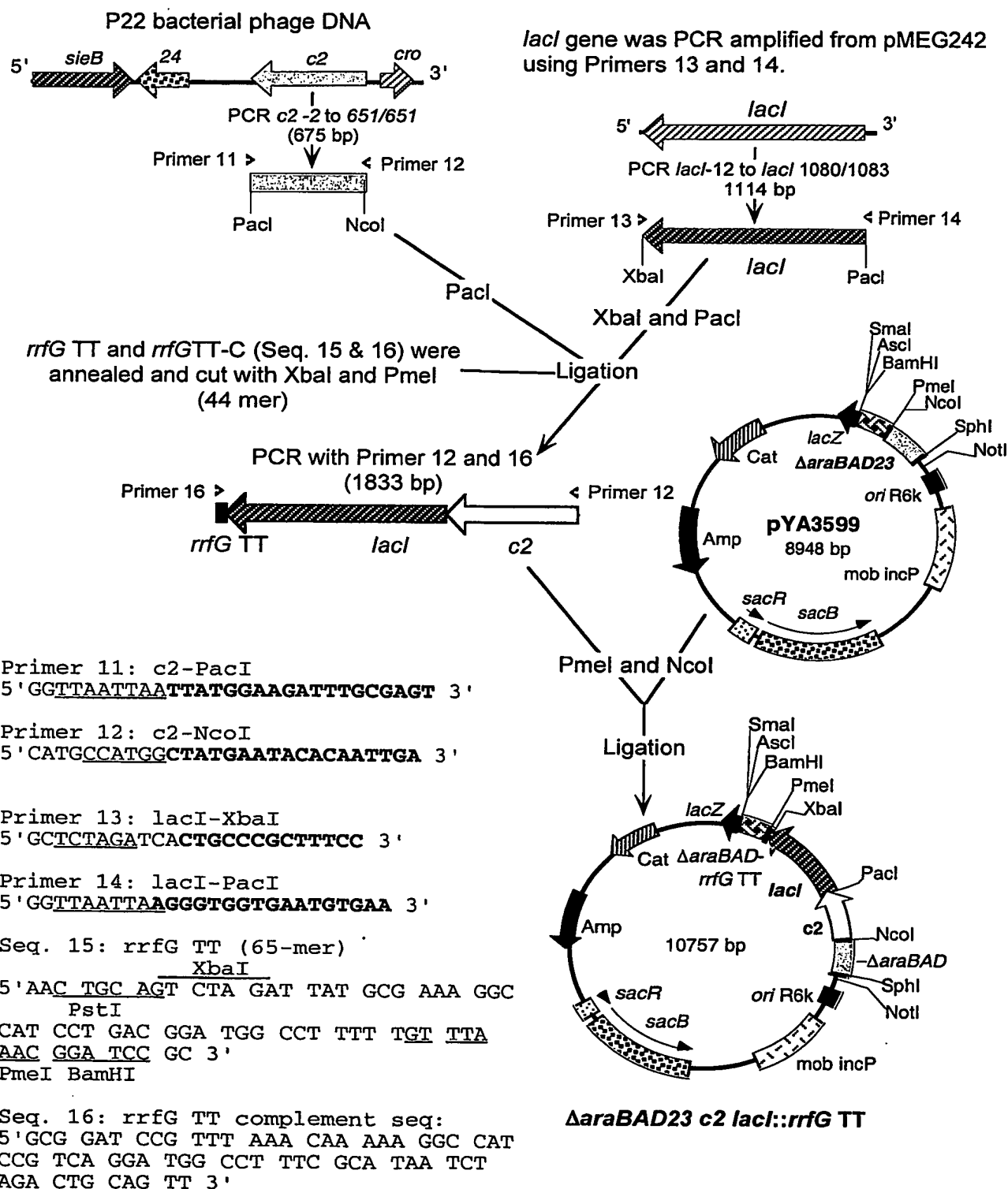


FIGURE 12. continued

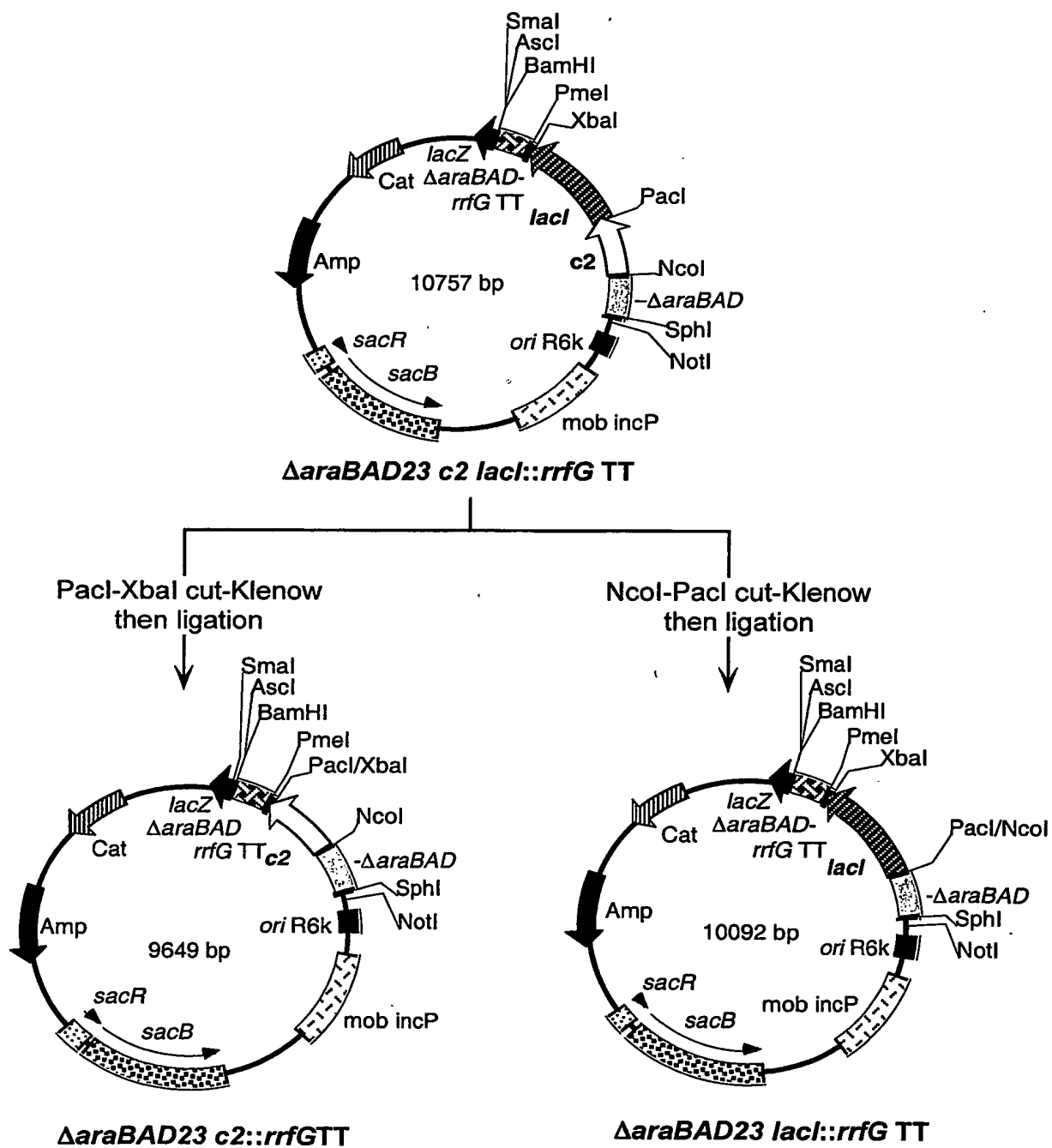




FIGURE 12. continued

In *S. typhimurium* chromosome:

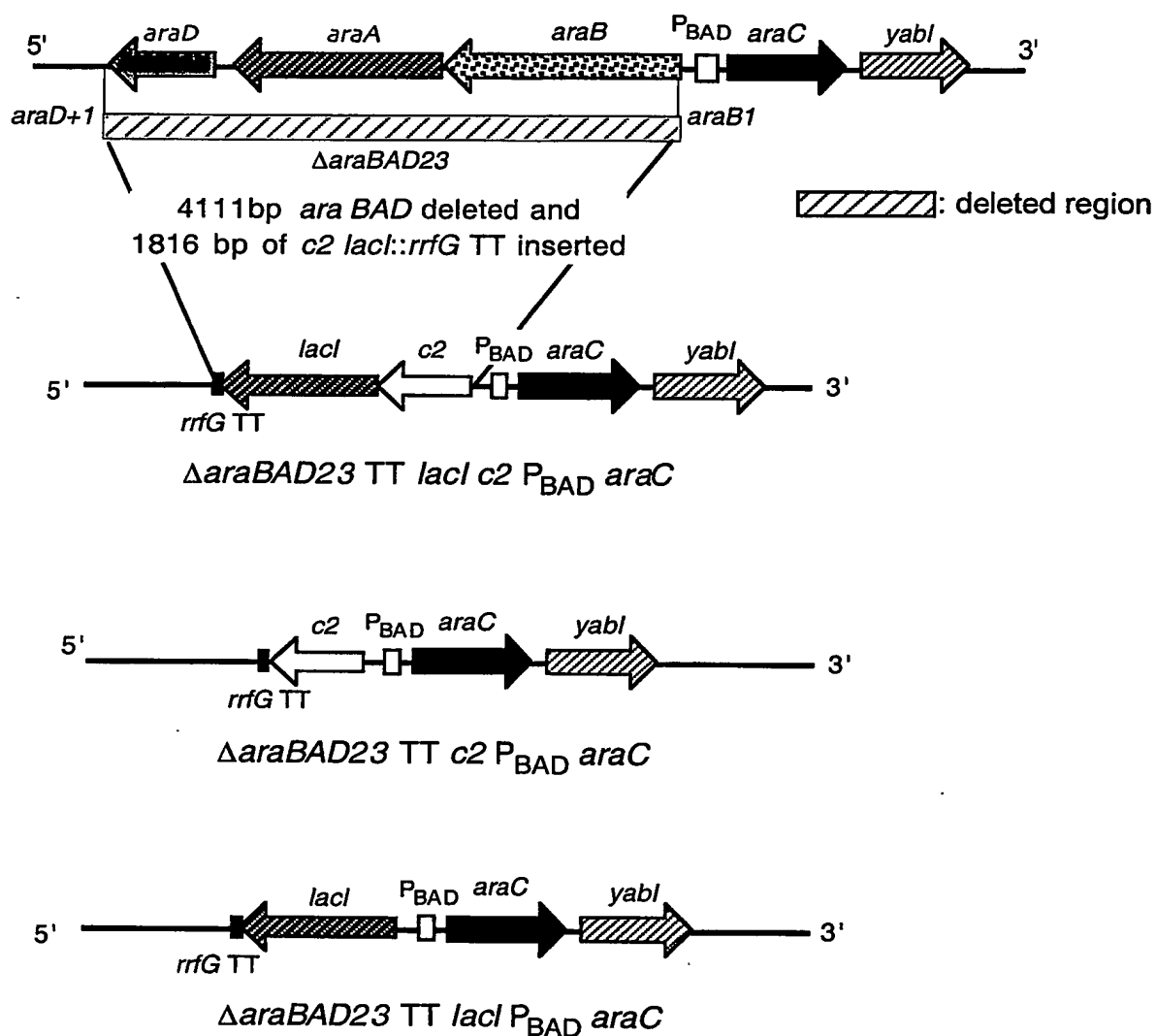
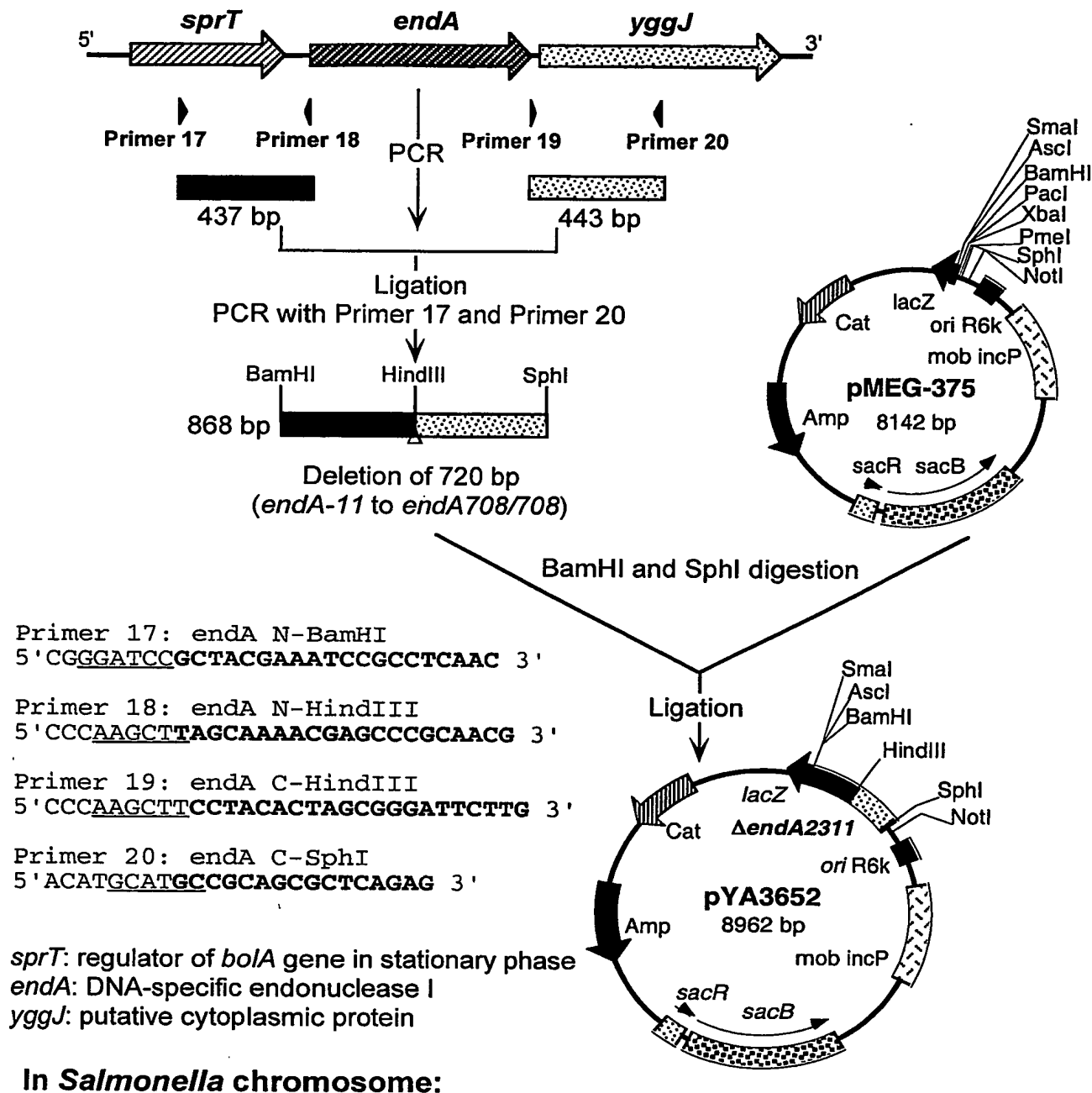


FIGURE 13. Construction of suicide vector for  $\Delta endA2311$ 

**FIGURE 14. Construction of suicide vector for  $\Delta endA23::TT$  *araCP<sub>BAD</sub> lacI* with improved *lacI* expression**

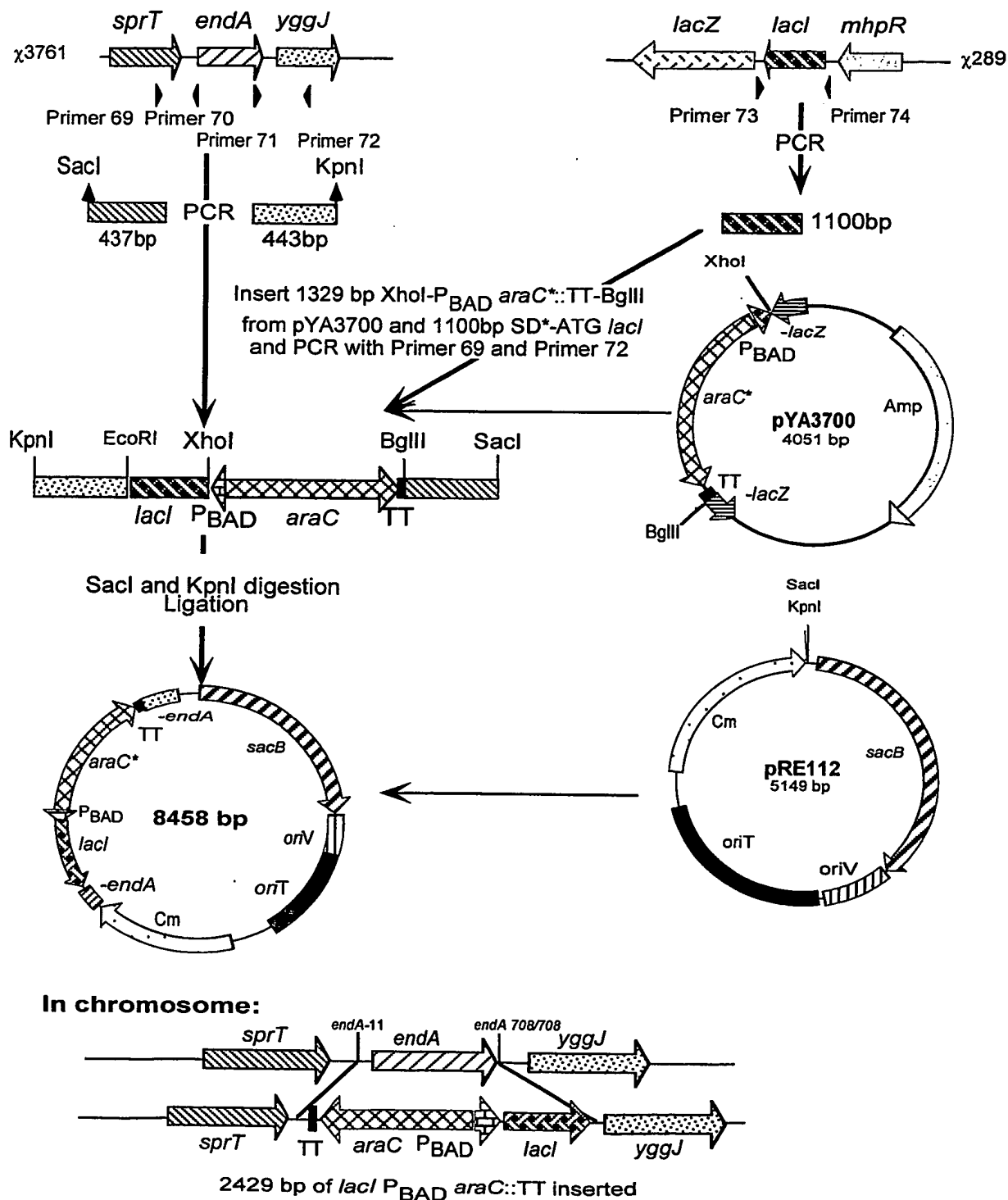
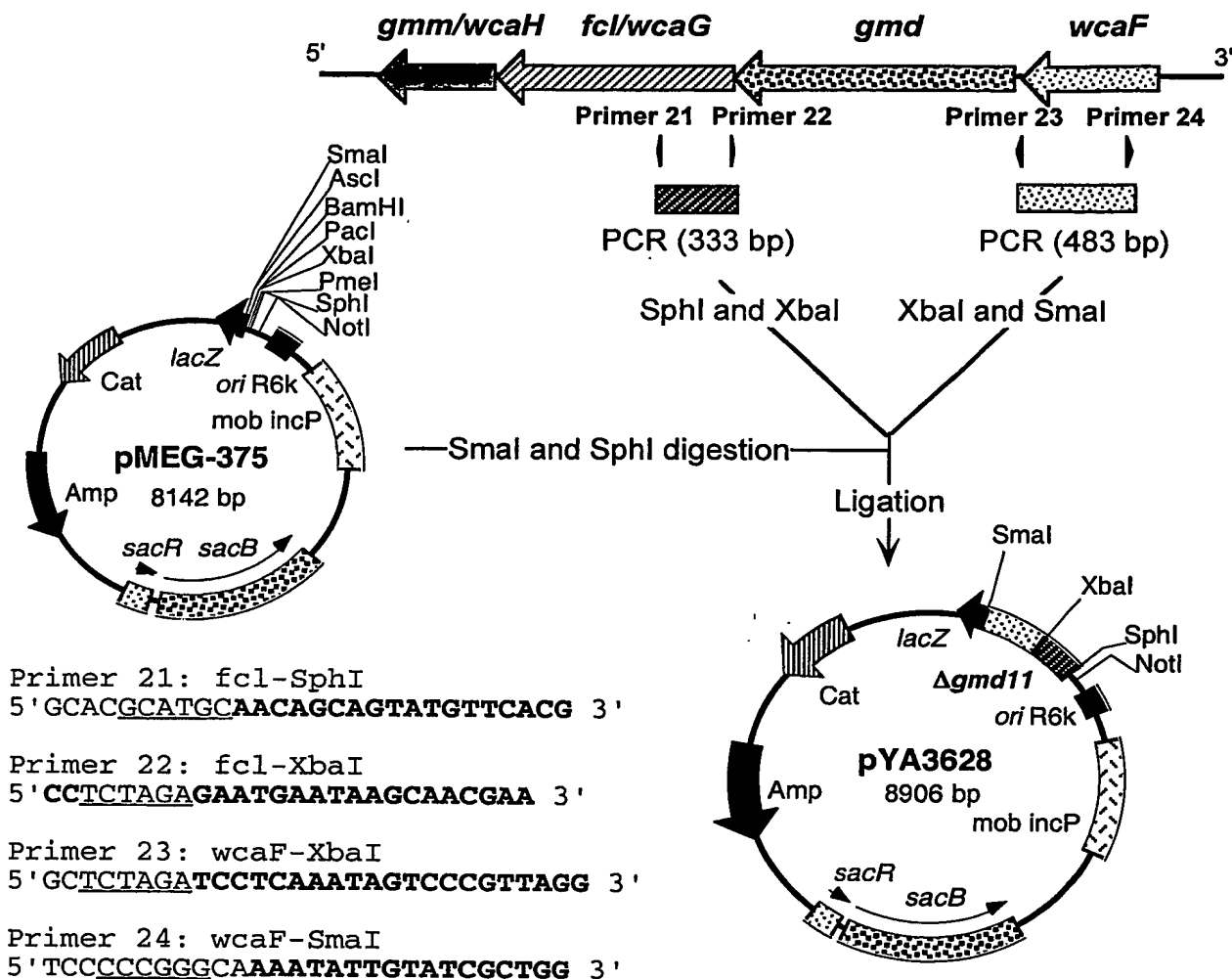


FIGURE 15. Construction of suicide vector for  $\Delta gmd-11$ 

*gmm/wcaH*: Guanosine di-P mannose mannosyl hydrolase

*fcl/wcaG*: Colanic acid gene cluster, bifunctional GDP fucose synthetase

*gmd*: Fucose biosynthesis; GDP-D-mannose 4,6-dehydratase

*wcaF*: Involved in lipopolysaccharide biosynthesis, putative acyltransferase

#### In *Salmonella* chromosome:

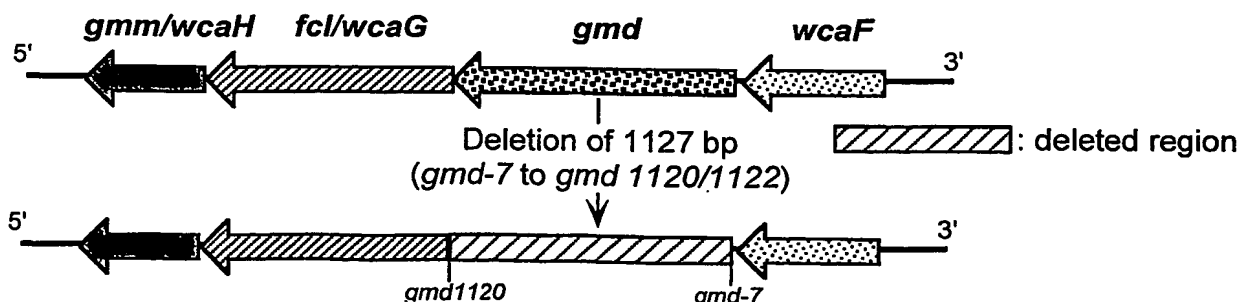
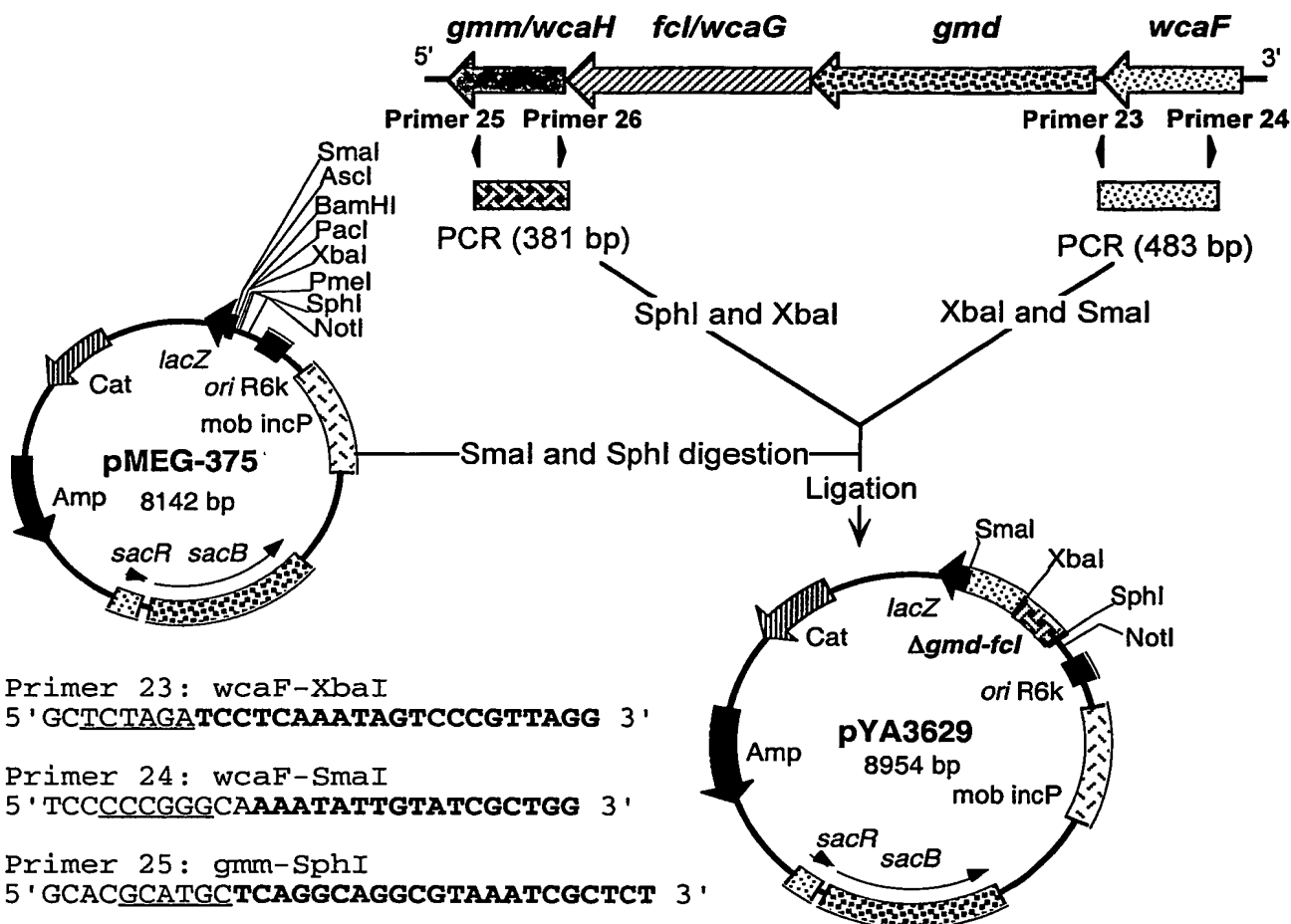


FIGURE 16. Construction of suicide vector for  $\Delta(gmd-fcl)$ -26

*gmm/wcaH*: Guanosine di-P mannose mannosyl hydrolase  
*fcl/wcaG*: Colanic acid gene cluster, bifunctional GDP fucose synthetase  
*gmd*: Fucose biosynthesis; GDP-D-mannose 4,6-dehydratase  
*wcaF*: Involved in lipopolysaccharide biosynthesis, putative acyltransferase

#### In *Salmonella* chromosome:

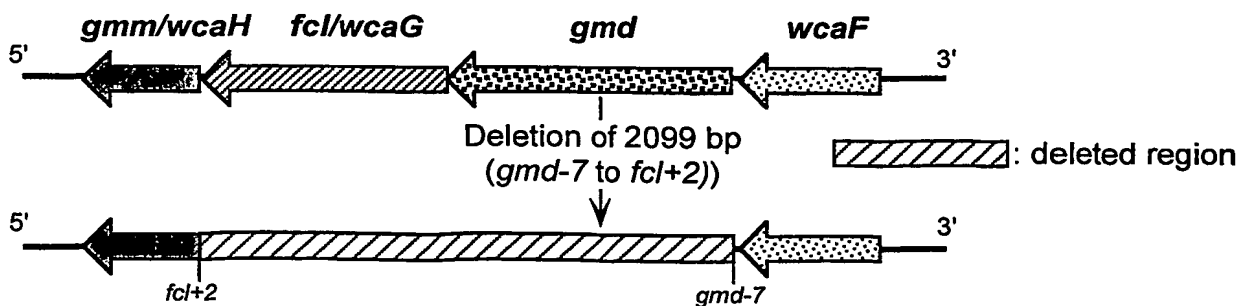
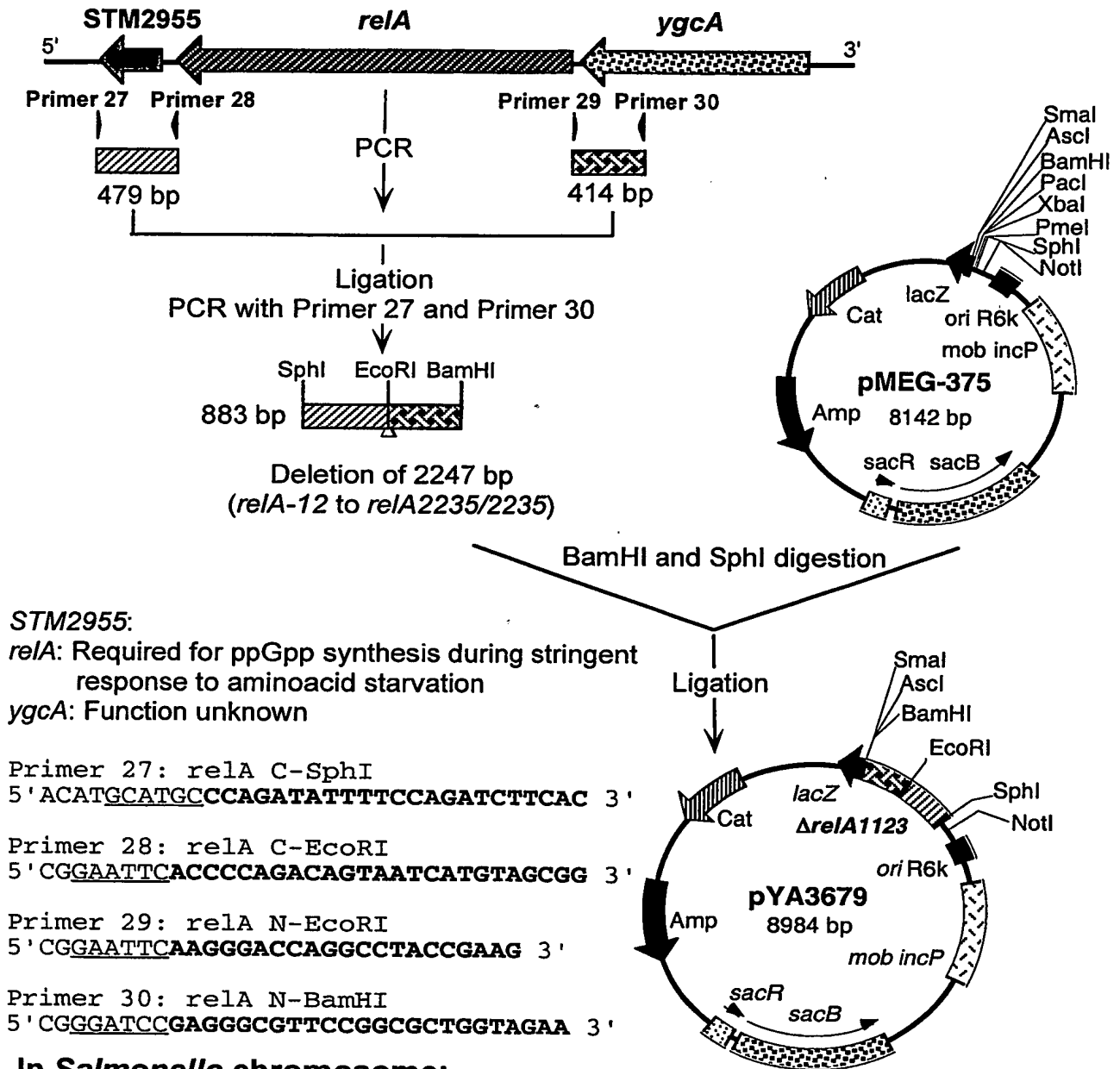
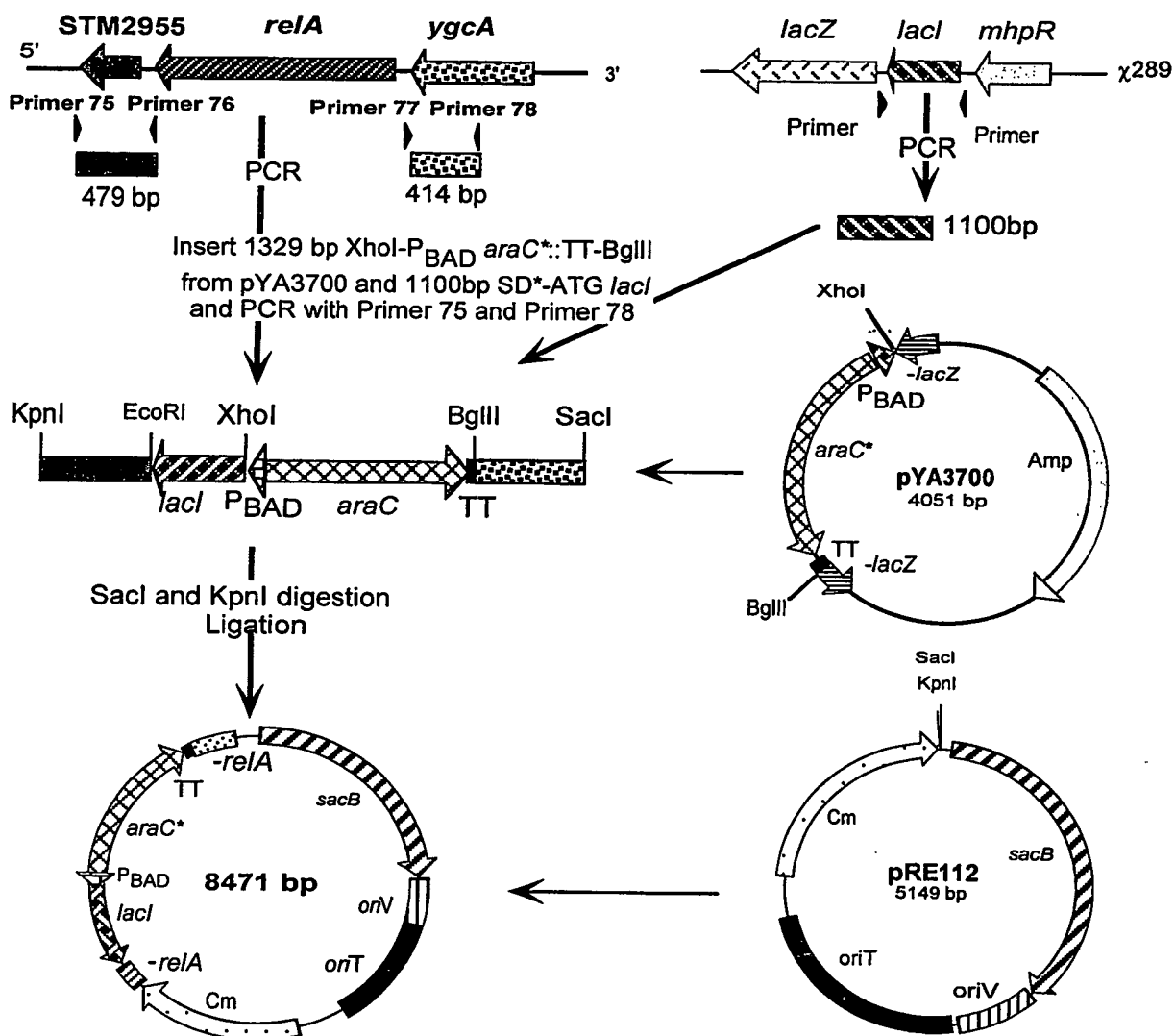
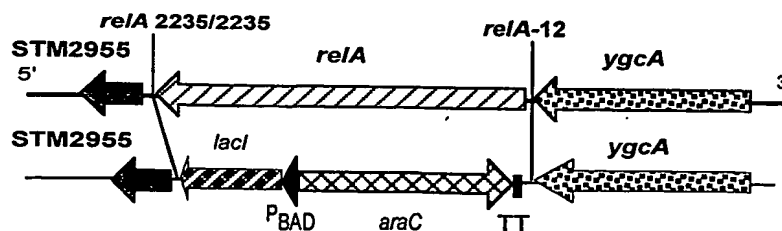


FIGURE 17. Construction of suicide vector for  $\Delta relA1123$ In *Salmonella* chromosome:

**FIGURE 18. Construction of suicide vector for  $\Delta relA11::TT$  *araCP<sub>BAD</sub> lacI* with improved *lacI* expression**

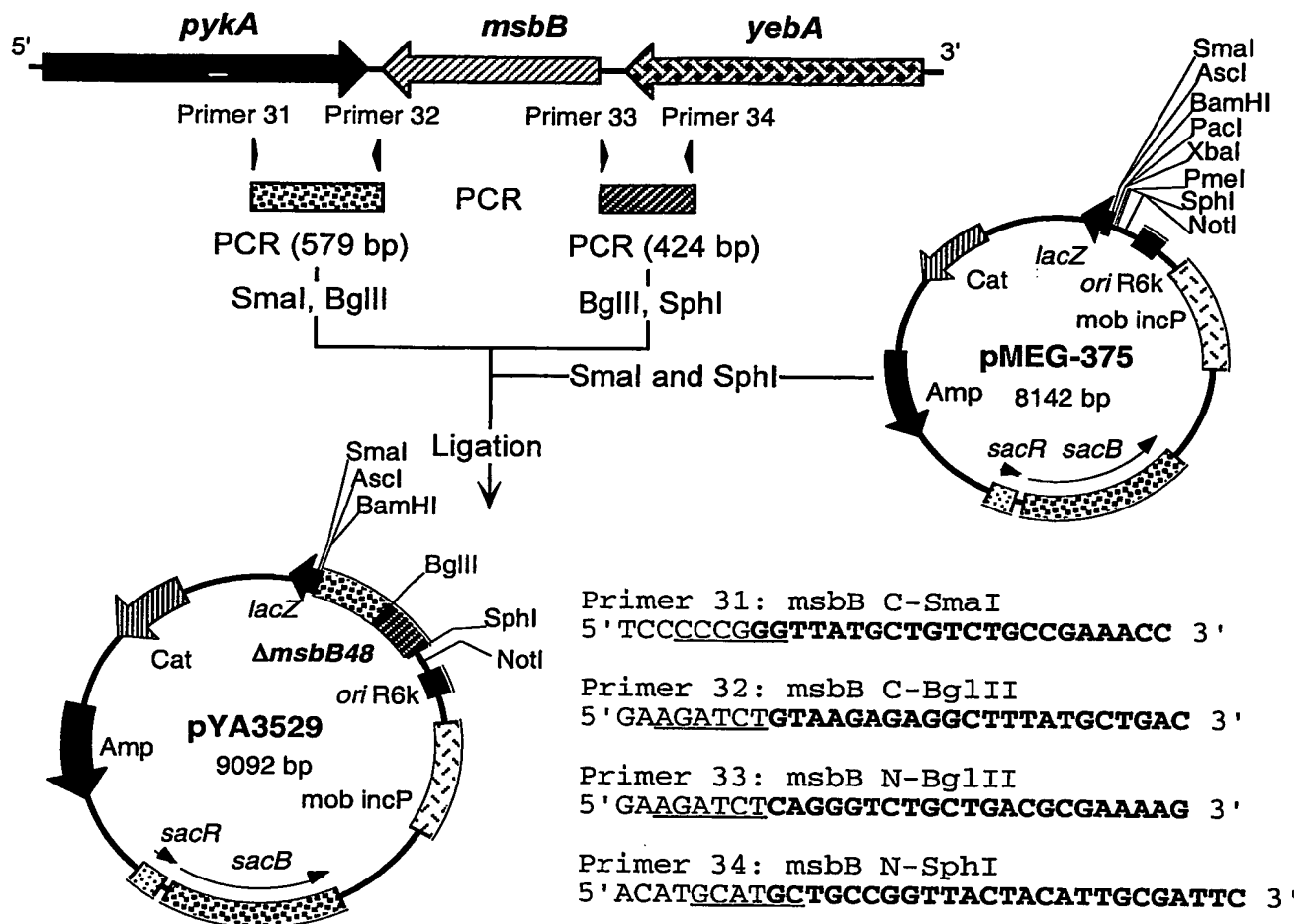


**In chromosome:**



Deletion of 2247 bp (*relA*-12 to *relA*2235/2235) and inserted 2429 bp of *araCP<sub>BAD</sub> lacI*

**FIGURE 19. Construction of suicide vector for  $\Delta msbB48$**



*pykA*: Pyruvate kinase A (II)

*msbB*: Role in outer membrane structure; myristoyl transferase in lipid A biosynthesis

*yebA*: Putative peptidase

**In *Salmonella* chromosome:**

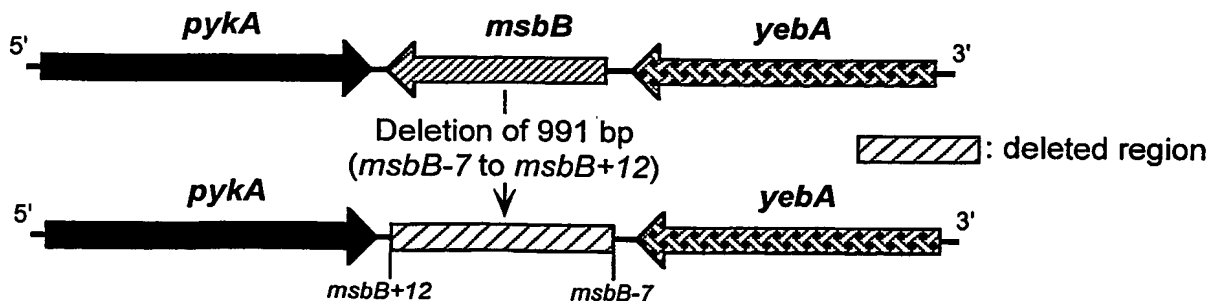




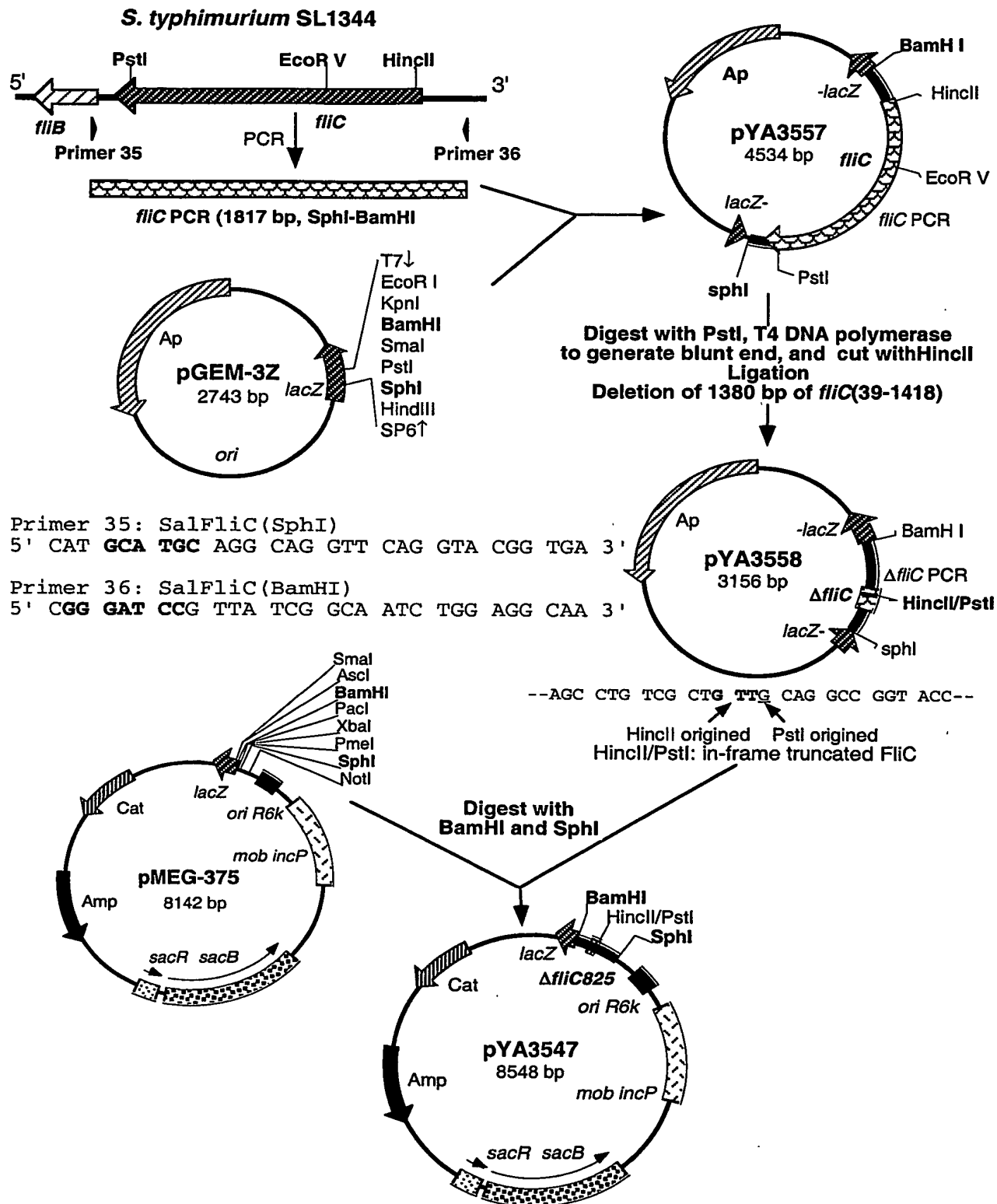
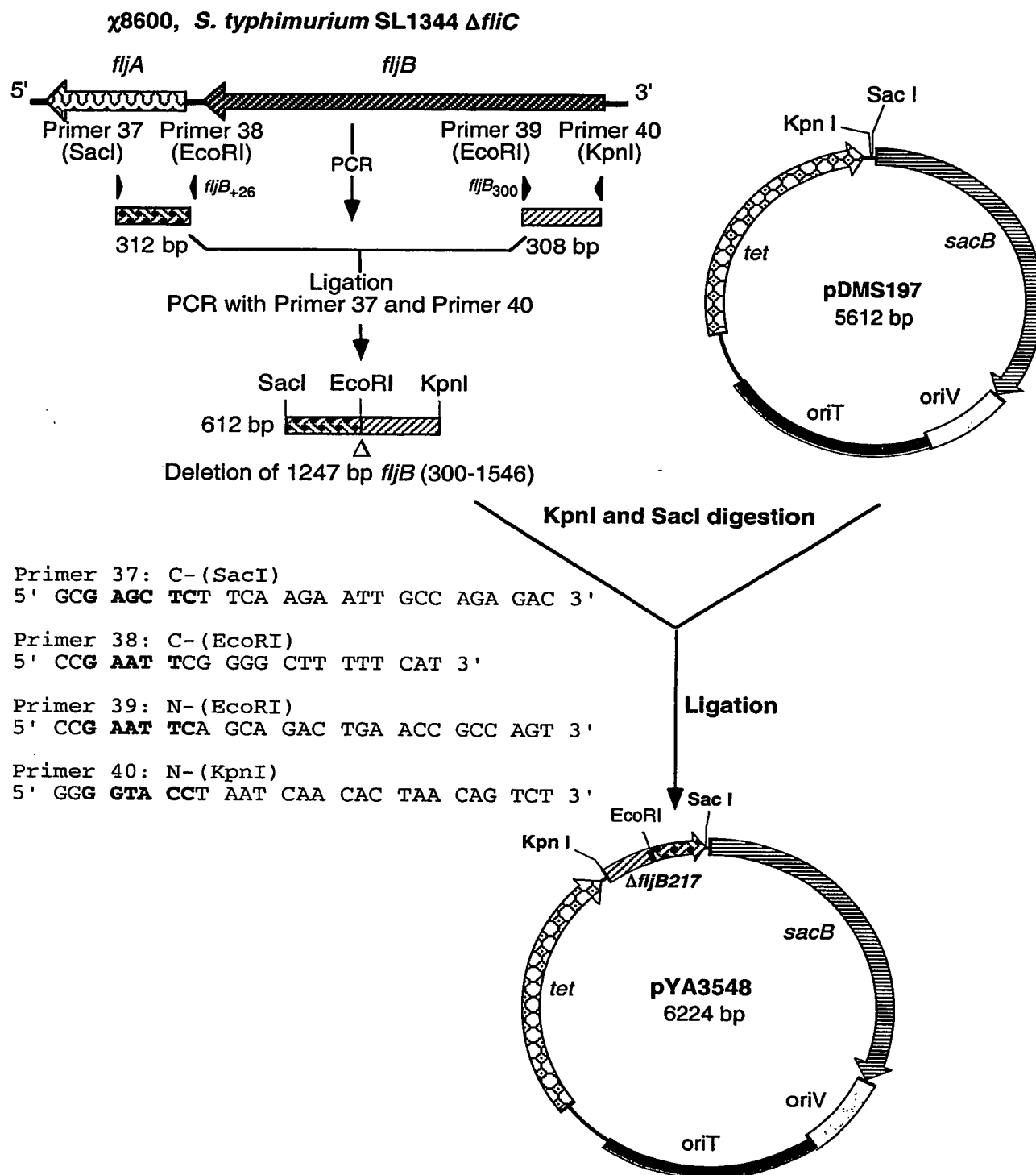
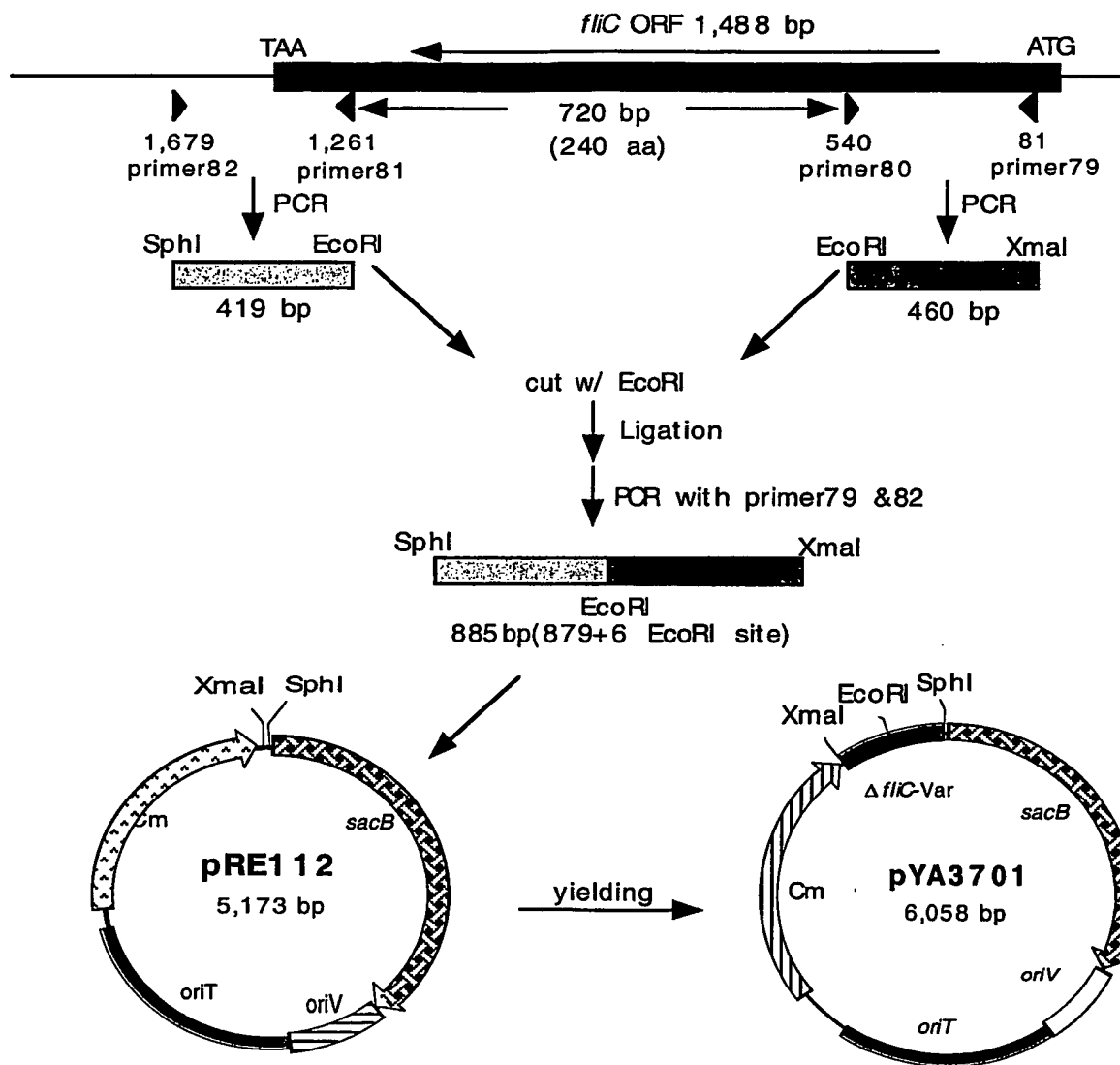
FIGURE 20. Construction of suicide vector for  $\Delta fliC825$ 

FIGURE 21. Construction of suicide vector for  $\Delta fljB217$ 

**Figure 22. Construction of a suicide vector for transfer of  $\Delta fliC$ -Var mutation**



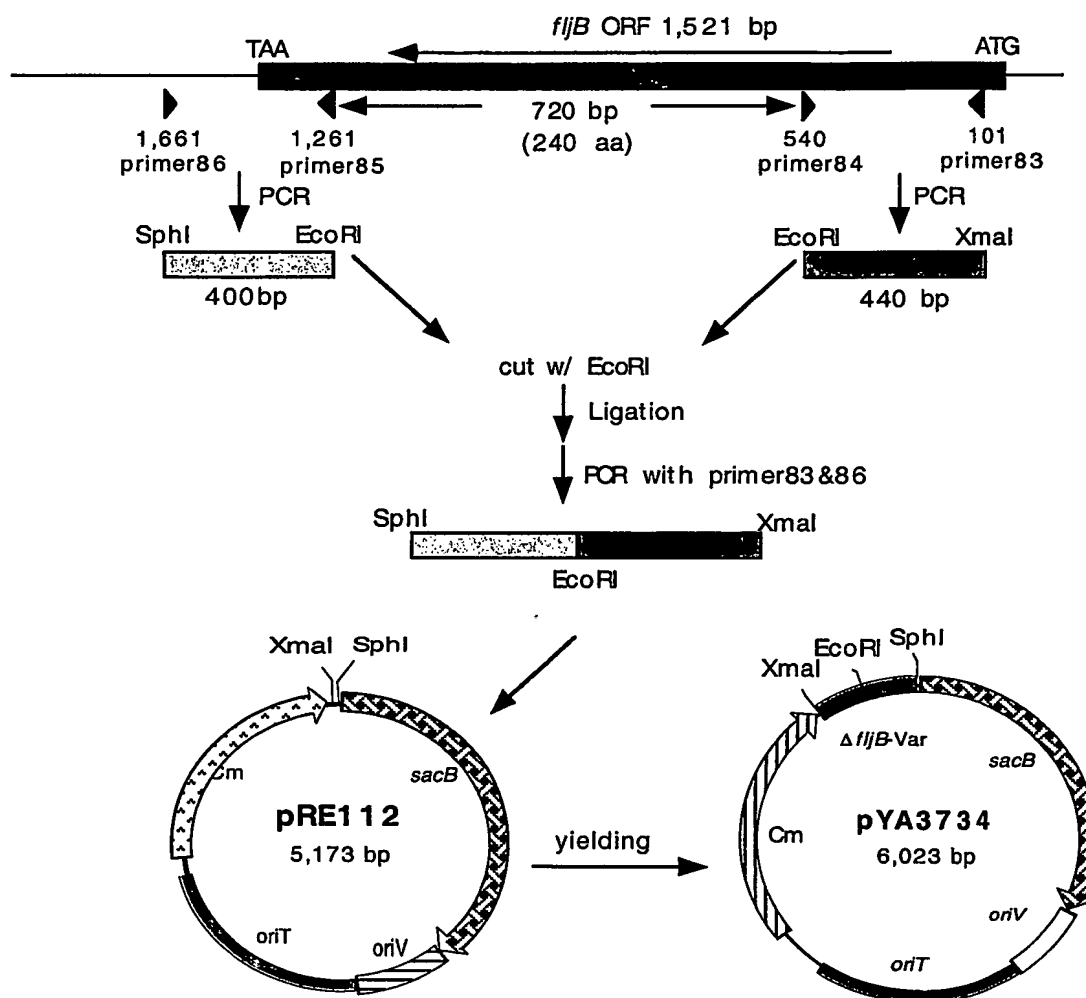
primer 79: delV.fliC 1 XmaI/bp81-104  
 5'-TCCOCCGGGGCTA TGG AGCGTCTGT CTT OCG G-3'

primer 80: delV fliC 2 EcoRI/bp540-516  
 5'-GGGAAT TOCTTATAT TTT TGT TGCACATTCAG-3'

primer 81: delV fliC 3 EcoRI/bp1261-1285  
 5'-GGGAAT TCA CGT TAC GTT CTGACCTGGGTGGG-3'

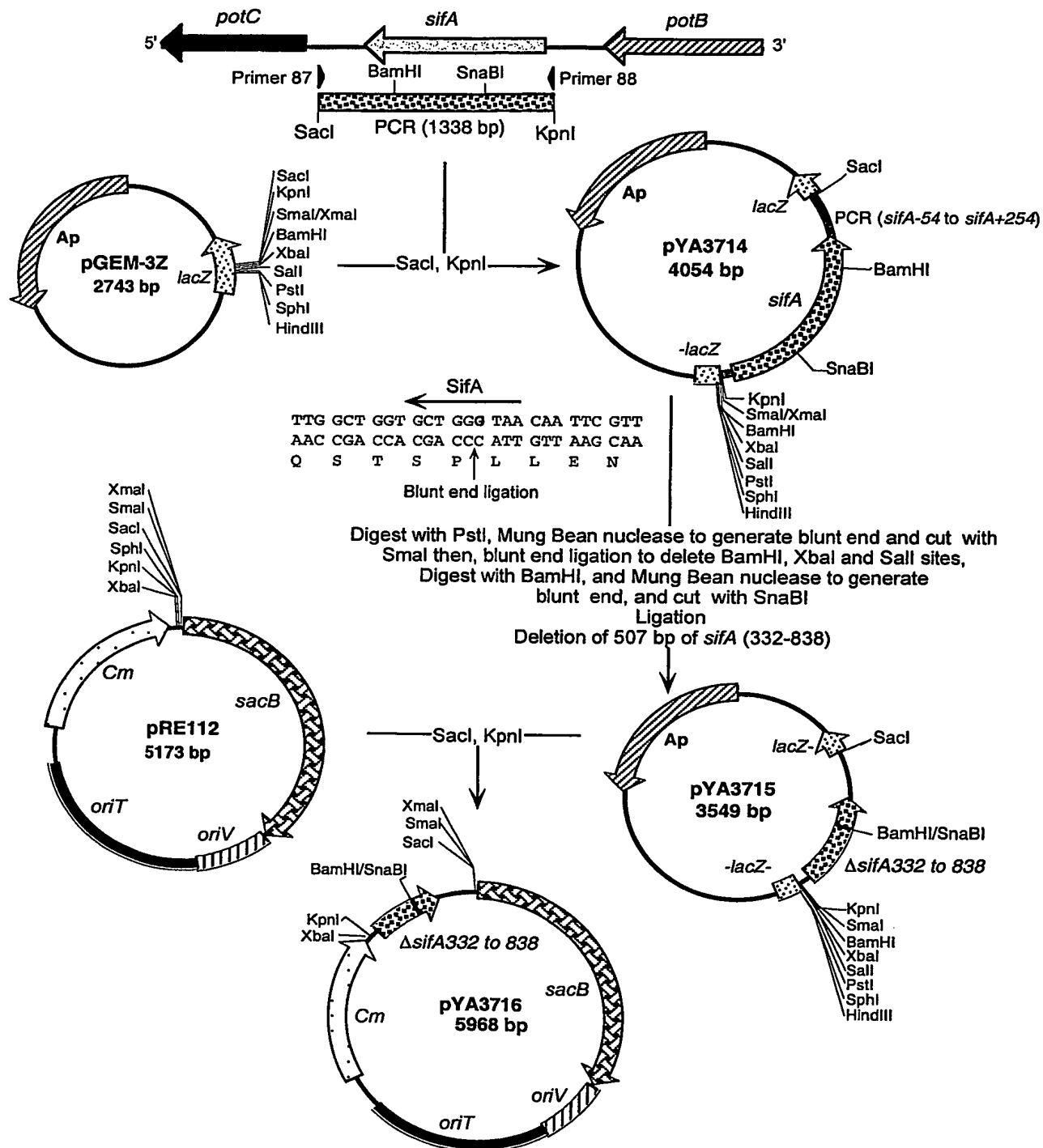
primer 82: delV fliC 4 SphI/bp1679-1655  
 5'-ACA TGCATGOOGTCTTAT CCAGCG TGA TTT TCCA-3'

**Figure 23. Construction of the suicide vector for the  $\Delta fliB$ -Var deletion mutation**



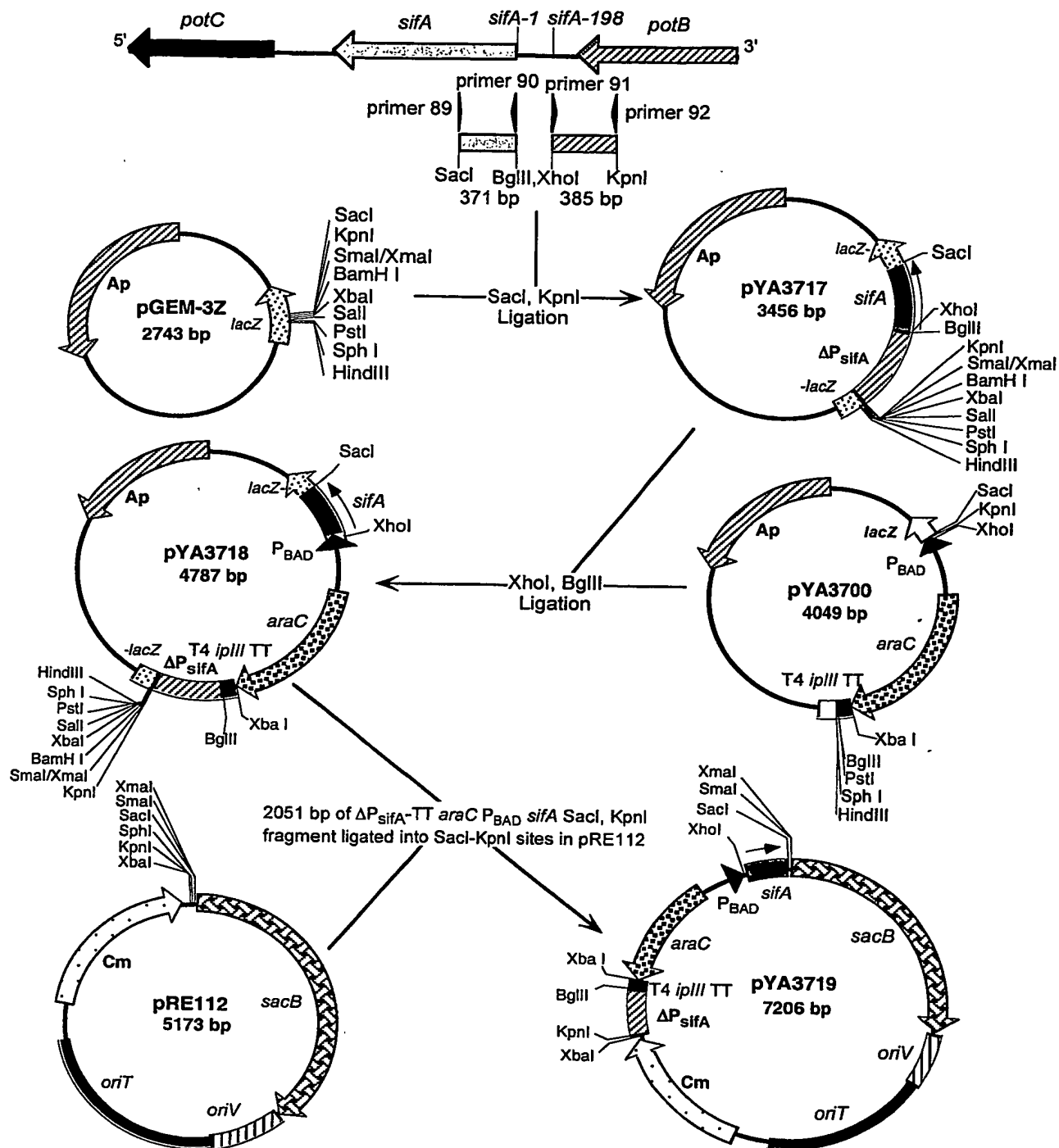
primer 83: delV.fliC 1 XmaI/bp81-104  
 5'-TCCCCCGGCT GGT CTGCGT ATCAAC AGC-3'  
 primer 84: delV fliC 2 EcoRI/bp540-516  
 5'-GGGAAT TCA TCA TAC GCT TTCTGCAOG TT-3'  
 primer 85: delV fliC 3 EcoRI/bp1261-1285  
 5'-GGGAAT TCCAGA AAA TTG ATGOOG CGCTG-3'  
 primer 86: delV fliC 4 SphI/bp1679-1655  
 5'-ACA TGCATG CCATAGA AAT AAT CCC GCG GCC-3'

**FIGURE 24. Construction of the suicide vector to make the  $\Delta sifA26$  (in-frame deletion) mutation**



Primer 87: TGATGAGCTCTTTCTCTTCTCCAAAATCTC (*sifA* *SacI*)  
 Primer 88: CTTAGGTACCGGTCGATTTAATCAATTATG (*sifA* *KpnI*)

FIGURE 25. Construction of suicide vector  $\Delta P_{sifA196}::TT\ araC\ P_{BAD}\ sifA$

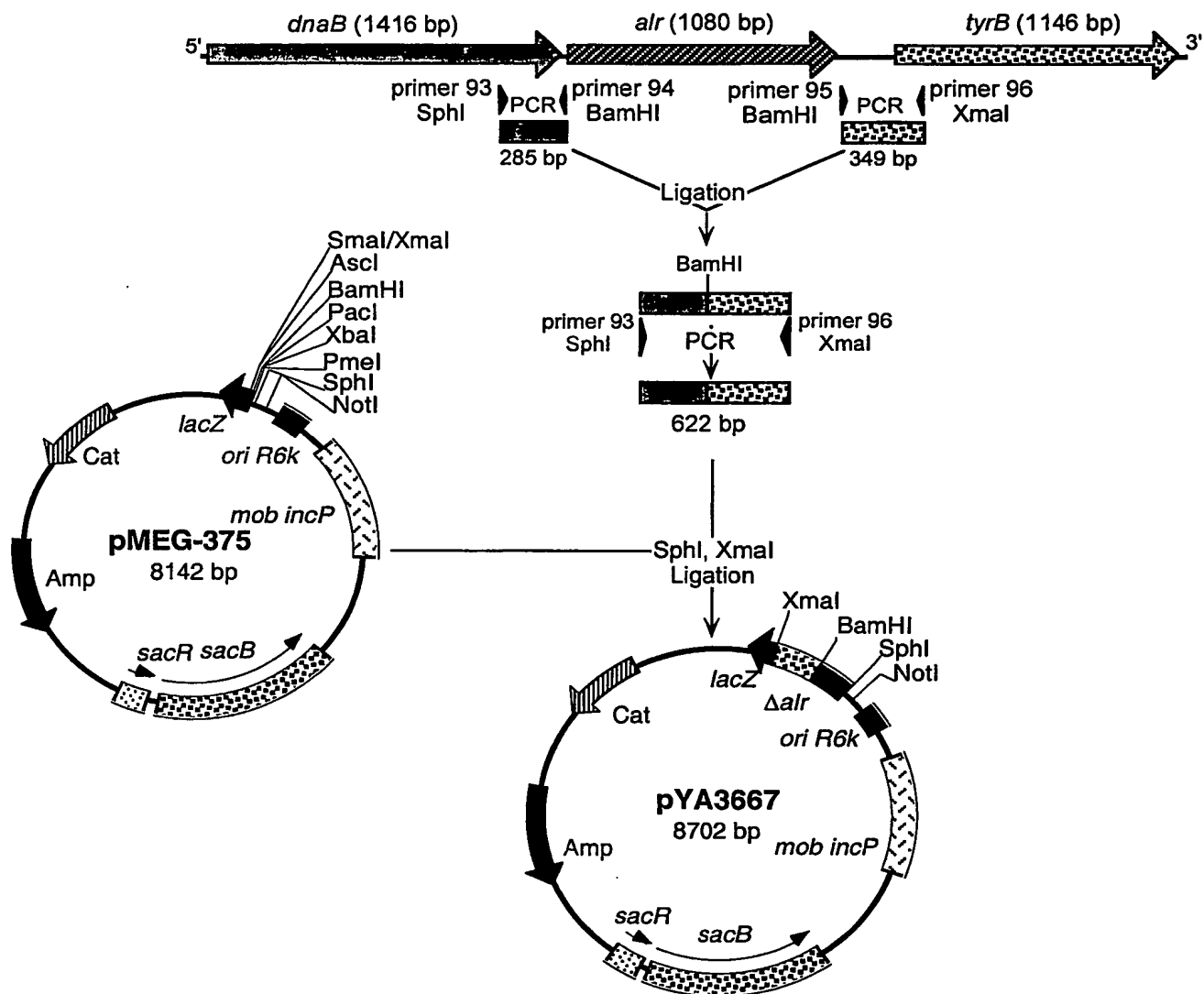


Primer 89: GCAAGAGCTCCTCTTCGTTTTGATCCATG (*sifA*-SacI)

Primer 90: GCCGGATCCAGATCTTATCTACTCGAGAGGAAAAACGCTATGCCGATTACTATAGGG (*sifA*-XhoI BglII)

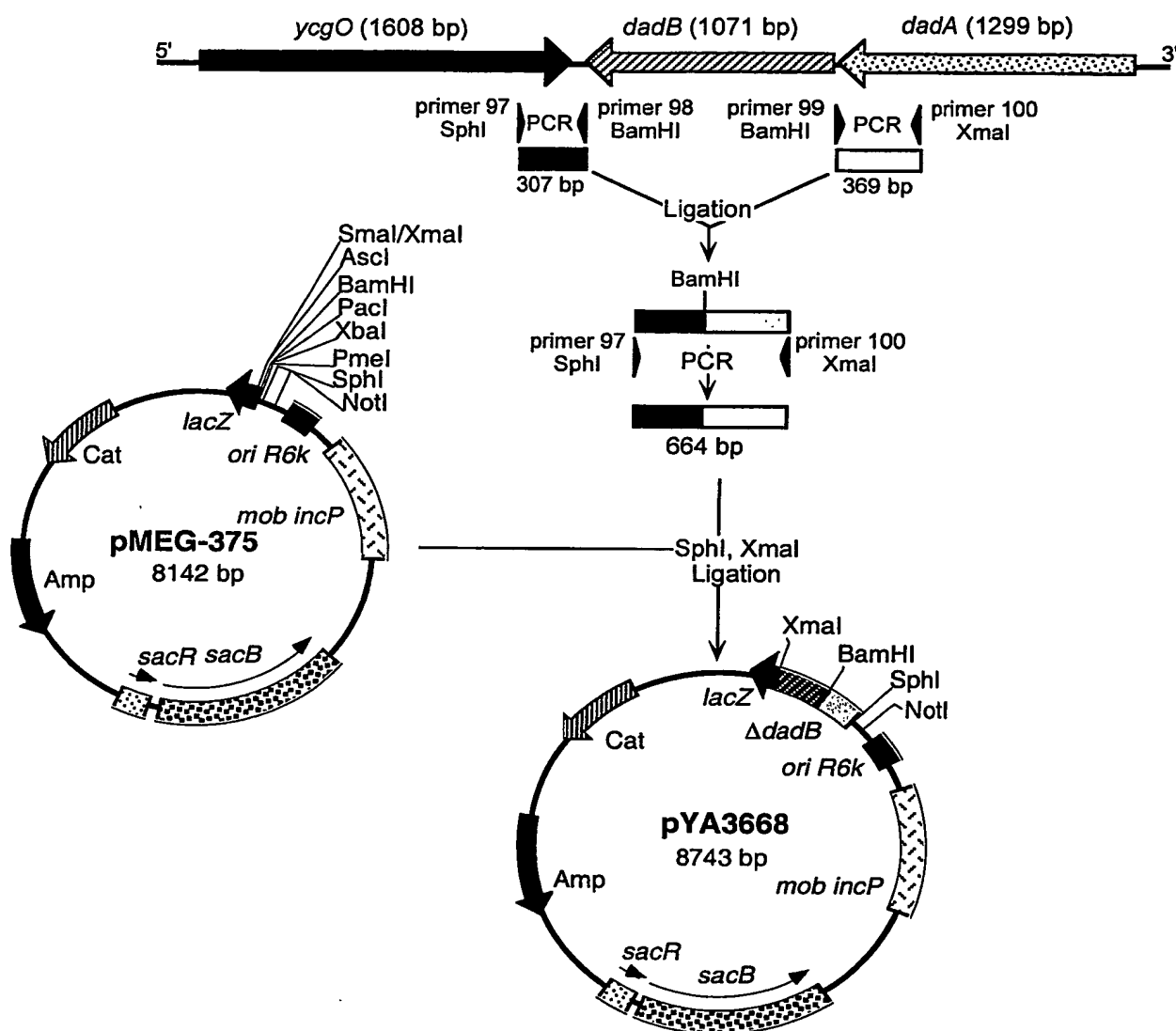
Primer 91: CCTCTCGAGTAGATAAGATCTGGATCCGGCGGATGATGTTGTAGATTG (*sifA*-XhoI BglII)

Primer 92: GCAGGTACCCGGCAATGGGCCTGTTCTAC (*sifA*-KpnI)

FIGURE 26. Construction of suicide vector with  $\Delta alr-3$  mutation

Primer 93: *dnaB*-SphI  
 ACATGCATGCCGCGCGGATAAACGTCCGGTGAAC  
 Primer 94: *dnaB*-BamHI  
 CGCGGATCCTGTTAAAGAATGACGGAGAGTTAC  
 Primer 95: *tyrB*-BamHI  
 CGTGGATCCGTGGCGCTTGCGCTTATCCGGCTTG  
 Primer 96: *tyrB*-XmaI  
 TCCCCGGGCTTCGGCTTCGGCCACCGTTTT

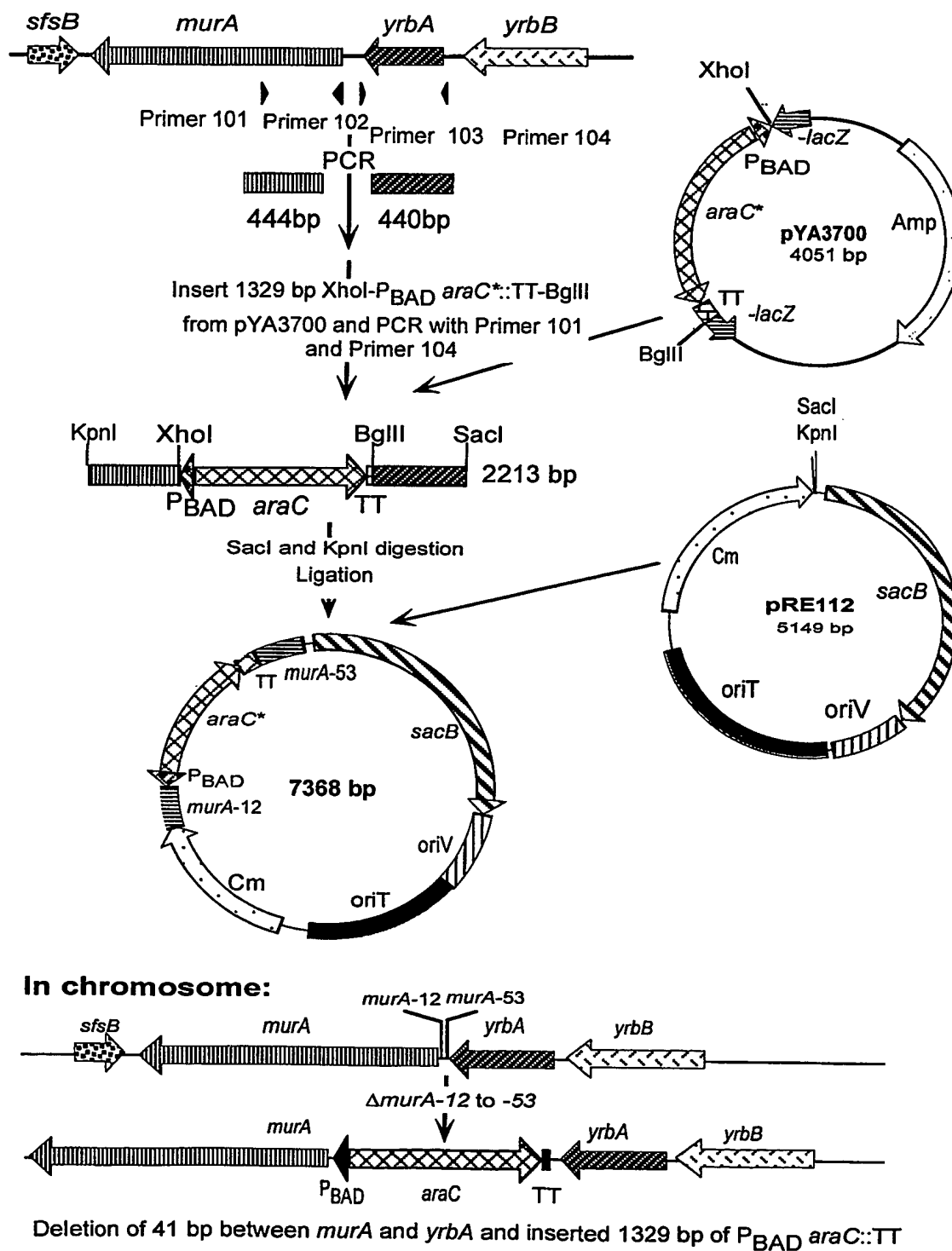
**FIGURE 27. Construction of suicide vector with  $\Delta dadB4$  mutation**

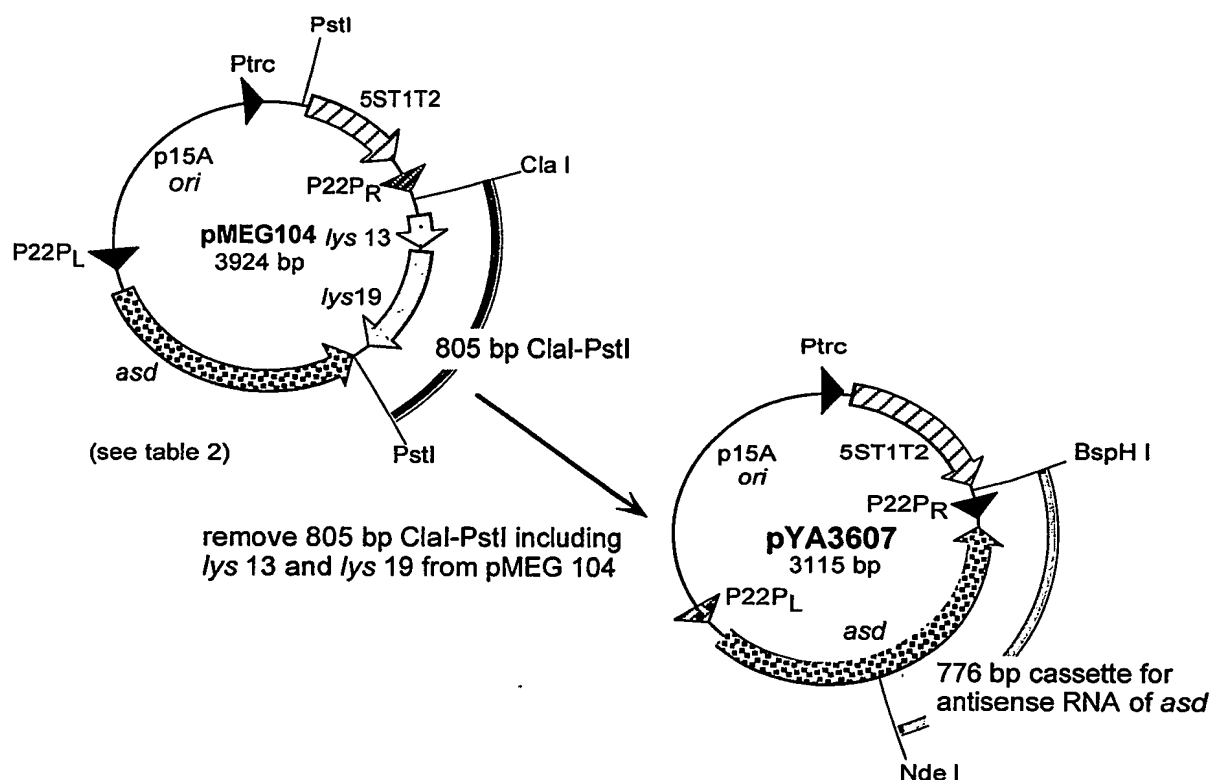


Primer 97: *ycgO*-SphI  
 ACATGCATGCGAATGCGAAATTCGCCGACGTG  
 Primer 98: *ycgO*-BamHI  
 CGCGGATCCTAATTCAGGCTAAGGCGTCGACC  
 Primer 99: *dadA*-BamHI  
 CGCGGATCCTTATCAGTTATGCGCGCTATGCAA  
 Primer 100: *dadA*-SmaI  
 TCCCCGGGCTTTAATACCGACTTACTGCAACC



**FIGURE 28. Construction of suicide vector with improved  $\Delta P_{mur::TT}$  *araC*  $P_{BAD}$  *murA* deletion-insertion mutation**



**FIGURE 29. Construction of pYA3607**

**Sequence of antisense RNA of *asd* from P22P<sub>R</sub> in pYA3607:**

TCATGAGACA	ATAACCCTGA	TAAATGCTTC	AATAATGGAA	GATCCTACGC	TCACCCATCA
BspH I					
ATTGTGTATT	CATAGTTAAG	TCATCTTAAA	TAAACTTGAC	TAAAGATTCC	TTTAGTAGAT
P22c2		OR3	OR2		
AATTTAAGTG	TTCTTTAAT	TCGGAGCGAG	TCTATGTACA	AGTCGACGGT	ATCGTGCAGC
OR1		P22cro			
TAGACTACGC	CAACTGGCGC	AGCATTCGAC	GCAGCGGCTC	GGCGGCGCCC	CATAACAAC
asd stop codon					
GGTCGCCTAC	GGTAAACGCC	GACAAGAACT	CTGGCCCCAT	GTTTCAGCTTA	CGCAGACGAC
asd					
CAACCGGCGT	AGTCAACGTG	CCGGTCACCG	CCGCCGGGGT	TAATTCGCGC	ATAGTGATAT
CACGATCGTT	CGGCACCACT	TTCGCCACG	GATTATGTGC	CGCCAGCAGT	TCTTCCACCG
TCGGAATGGA	TACCTCTTTT	TTCAGCTTGA	TGGTGAACGC	CTGGCTGTGA	CAGCGCAGCG
CGCCGACGCG	CACACACAAA	CCATCAACCG	GAATCACAGA	GGCAGTATTG	AGAATCTTGT
TGGTTTCCGC	CTGGCCTTTC	CACCTCTTCG	GGCTCTGGCC	GTTATCGAGC	TGTTTGTCGA
TCCAGGGGAT	CAGGCTTCCC	GCCAGCGGTA	CGCCAAAGTT	ATCAACCGGC	AGCTCGCCGC
TGCGGGTCAA	TGCCGTAAC	TTGCGTTCAA	TATCAAGAA	TGCGGAAGAC	GGCGTCGCCA
GTTTCATCGC	GACATGGCCA	TACAACTGAC	CCATCTGGGT	TAACAGCTCG	CGCATATG
asd					Nde I

**FIGURE 30. Construction of regulatable lysis system vector pYA3646:**

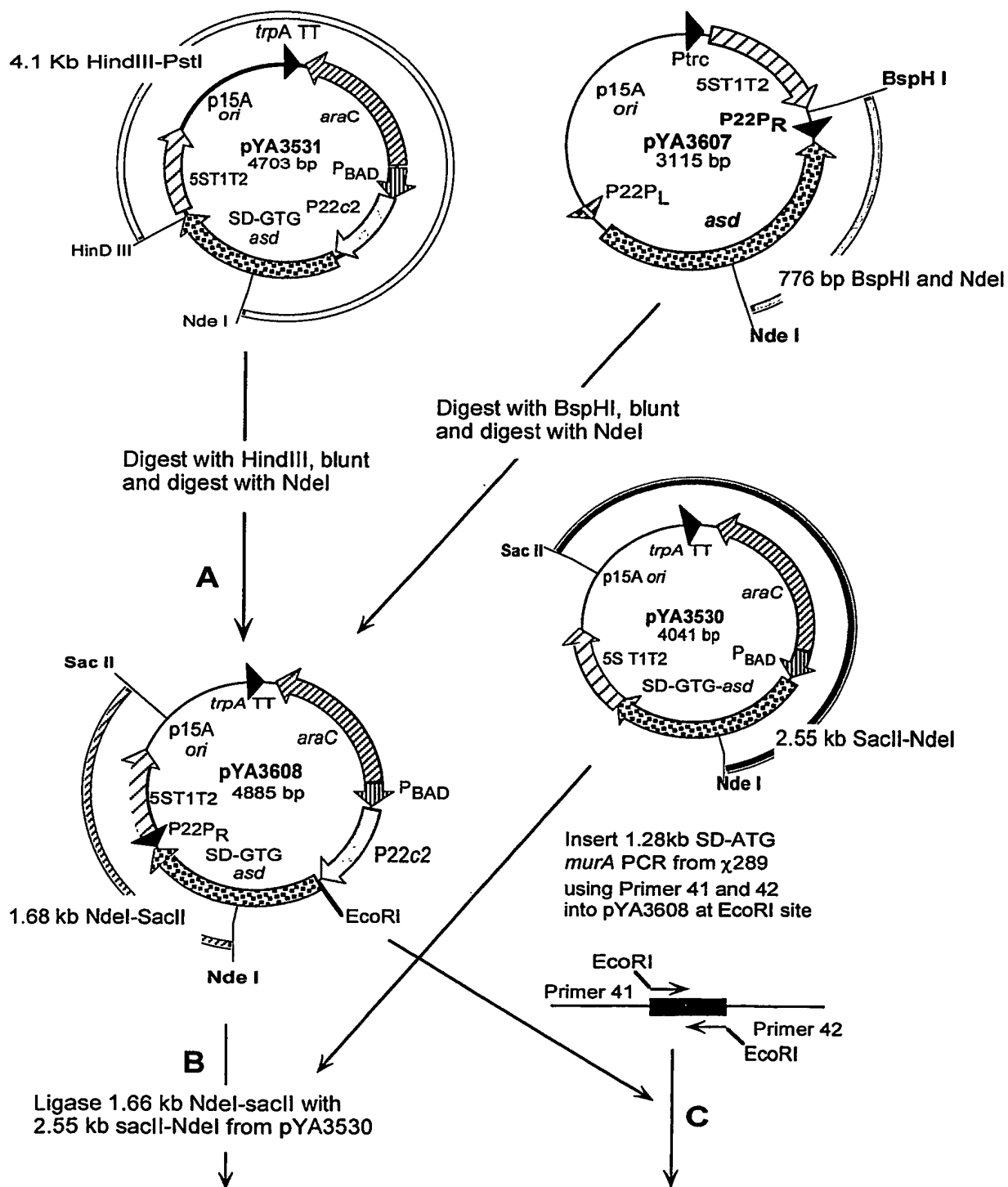
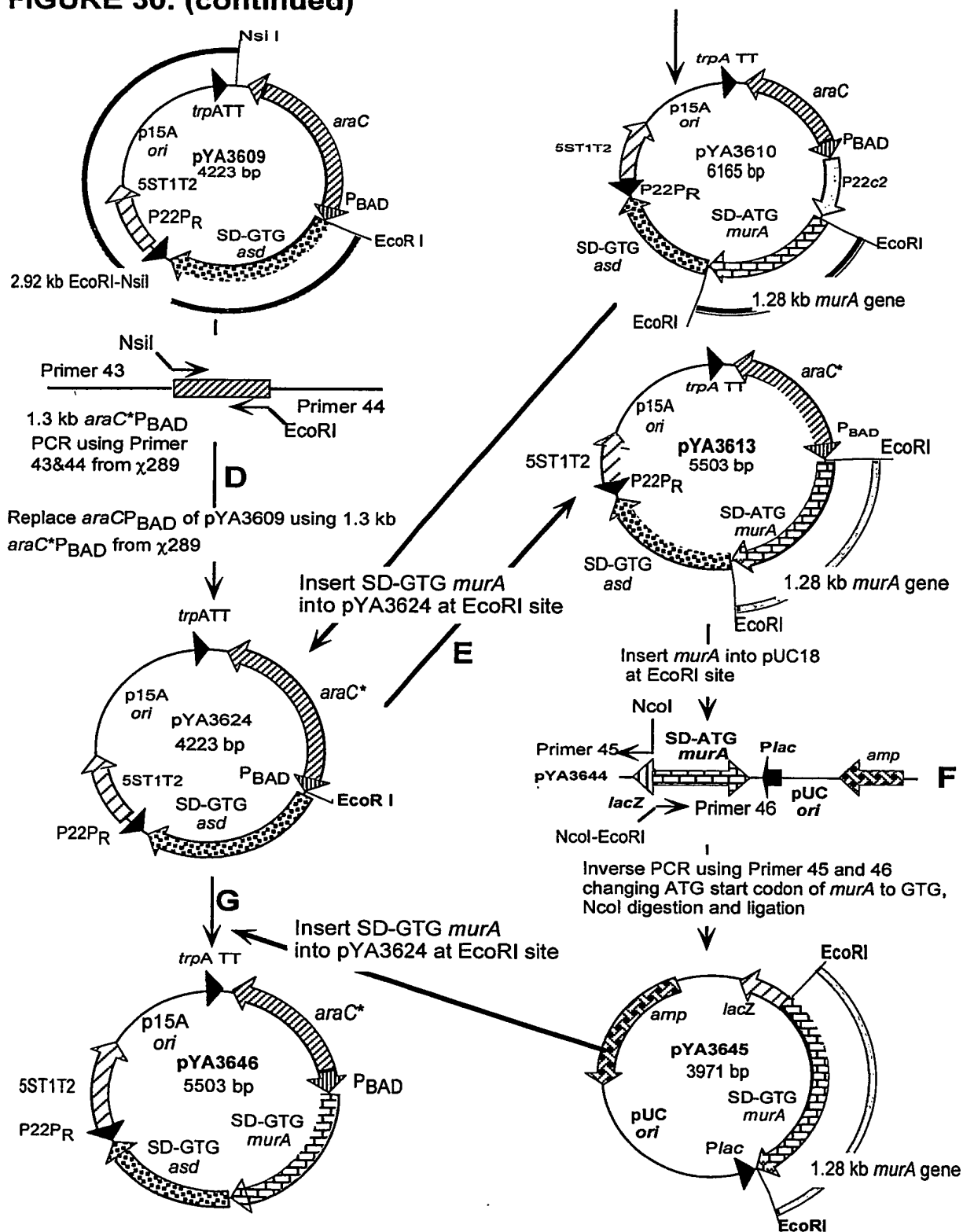
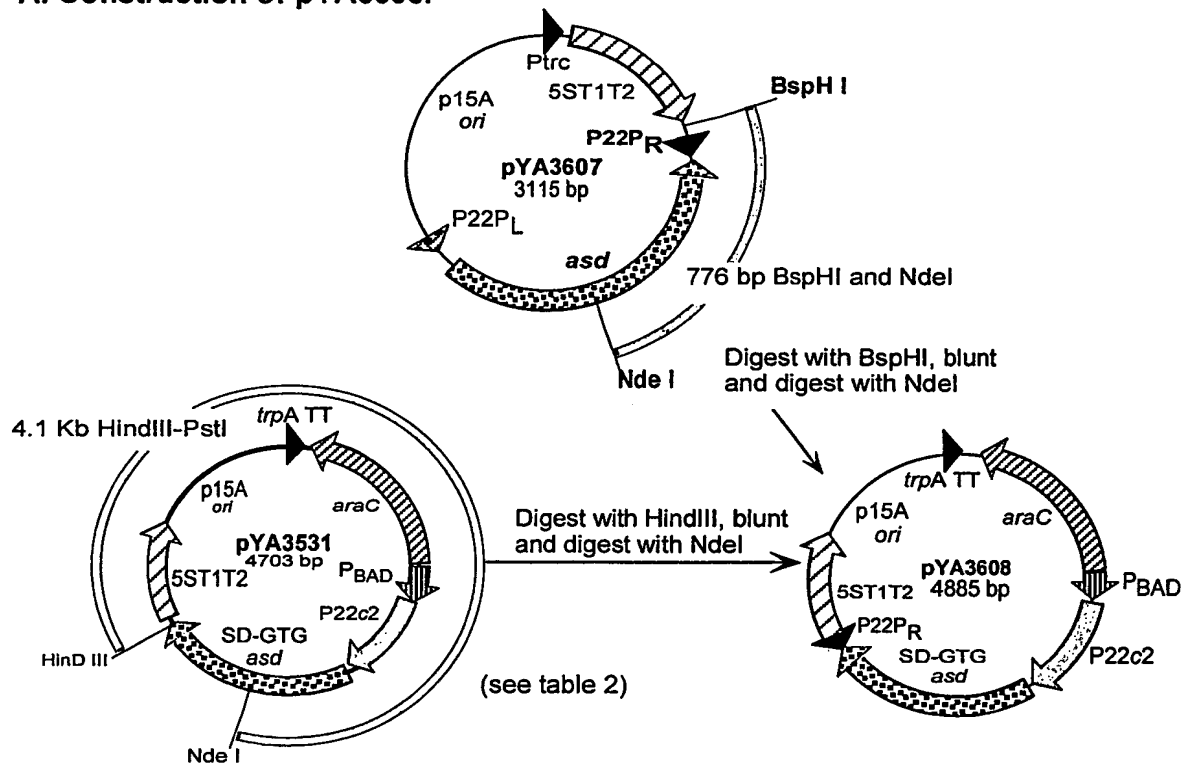


FIGURE 30. (continued)



**FIGURE 31. Steps in the construction of pYA3646**

**A. Construction of pYA3608.**



**B. Construction of pYA3609.**

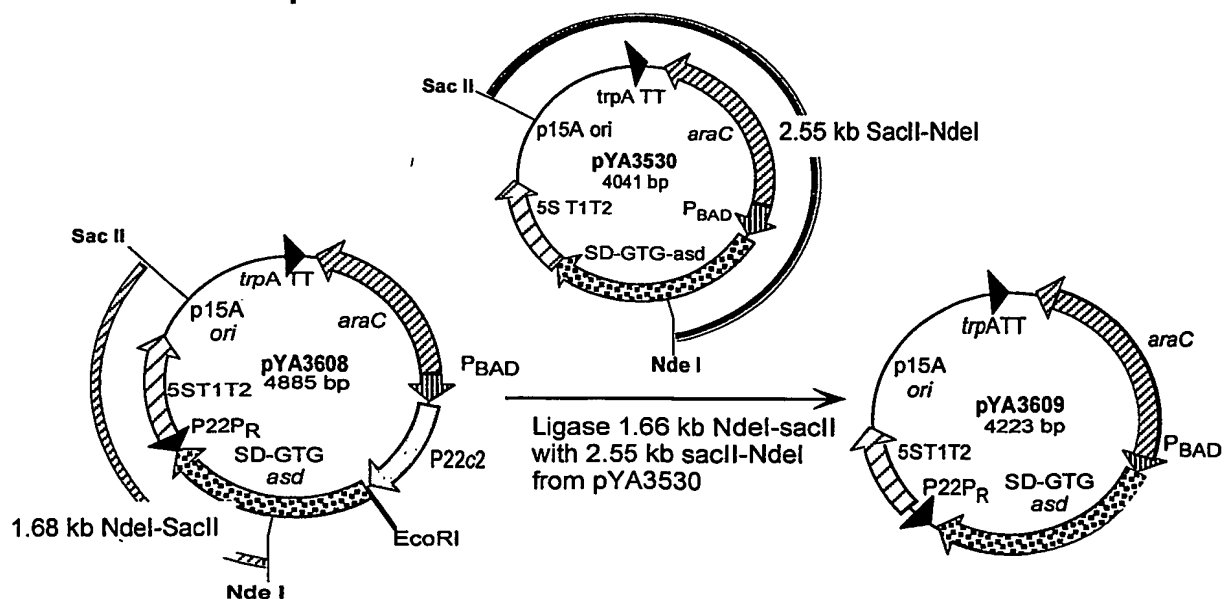
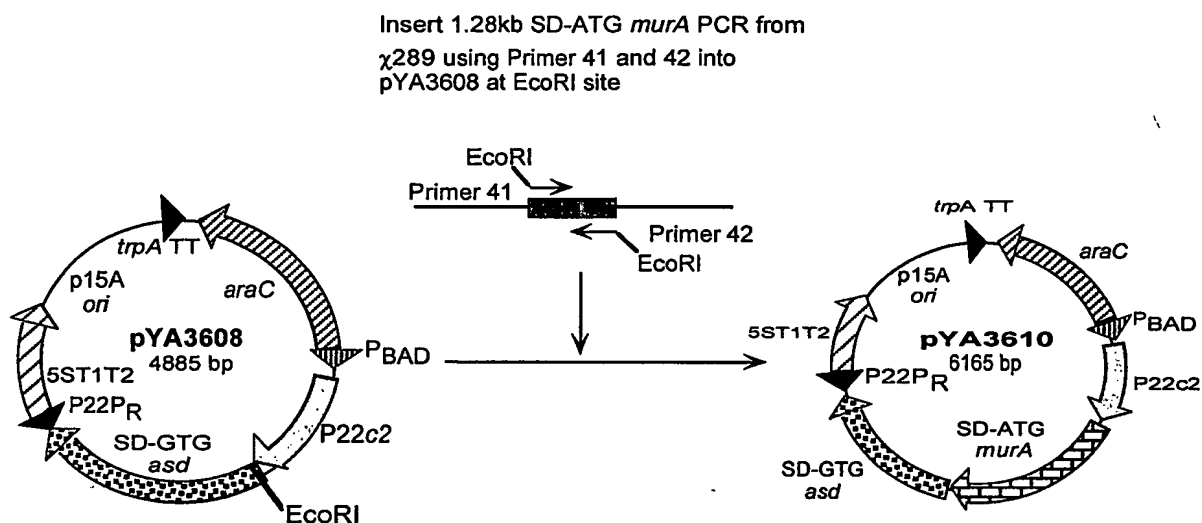


FIGURE 31. (continued)

## C. Construction of pYA3610.



## D. Construction of pYA3624.

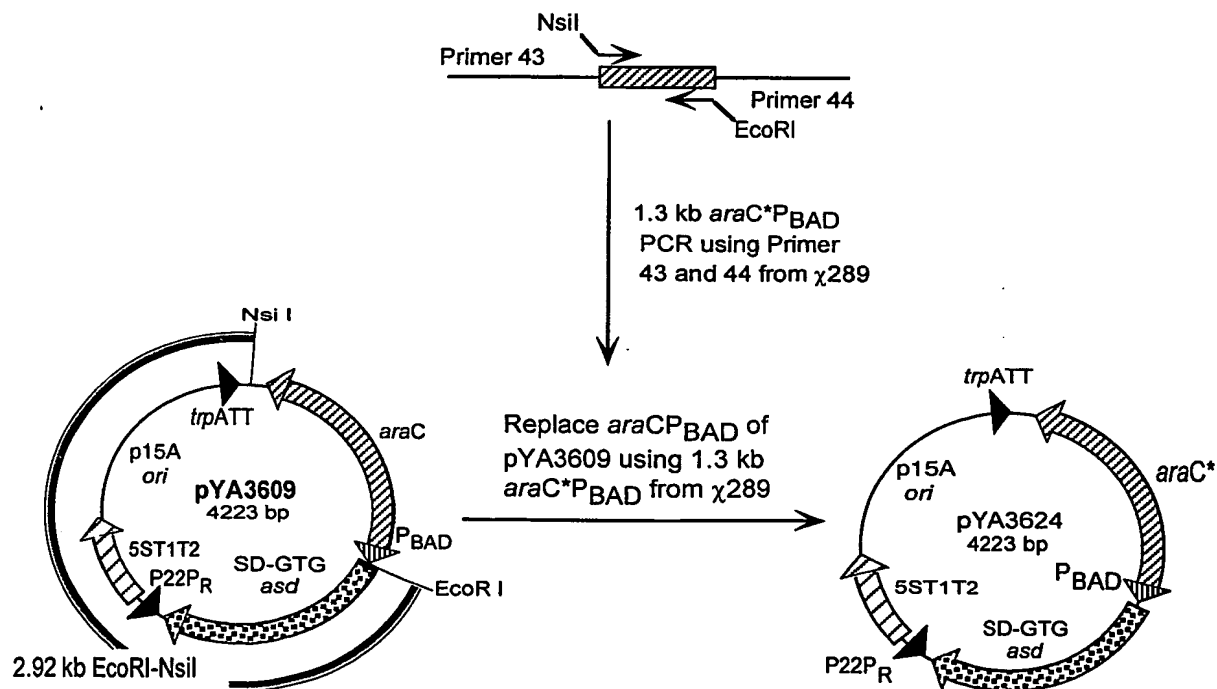
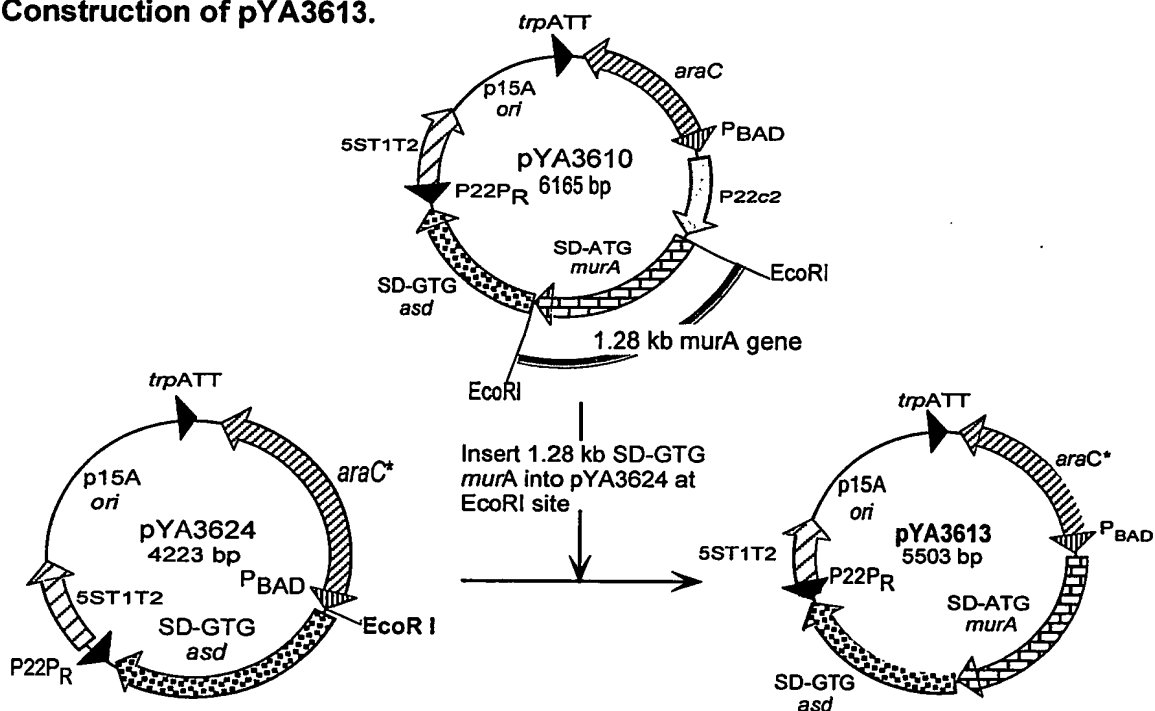


FIGURE 31. (continued)

## E. Construction of pYA3613.



## F. Construction of pYA3645.

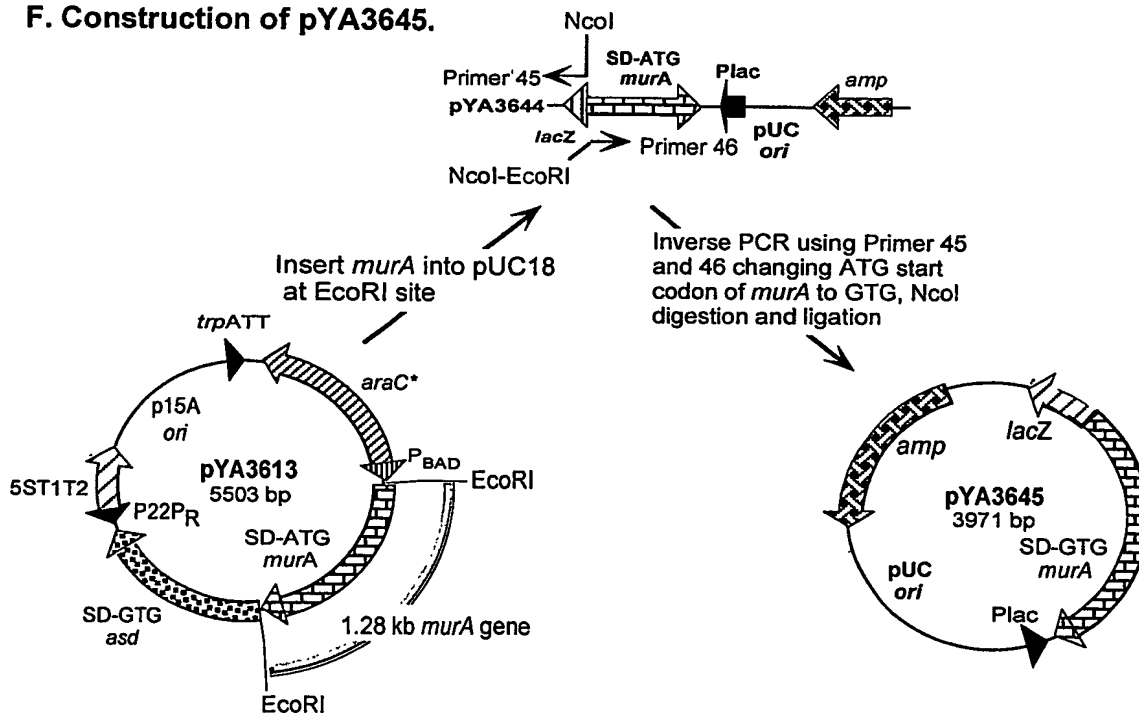
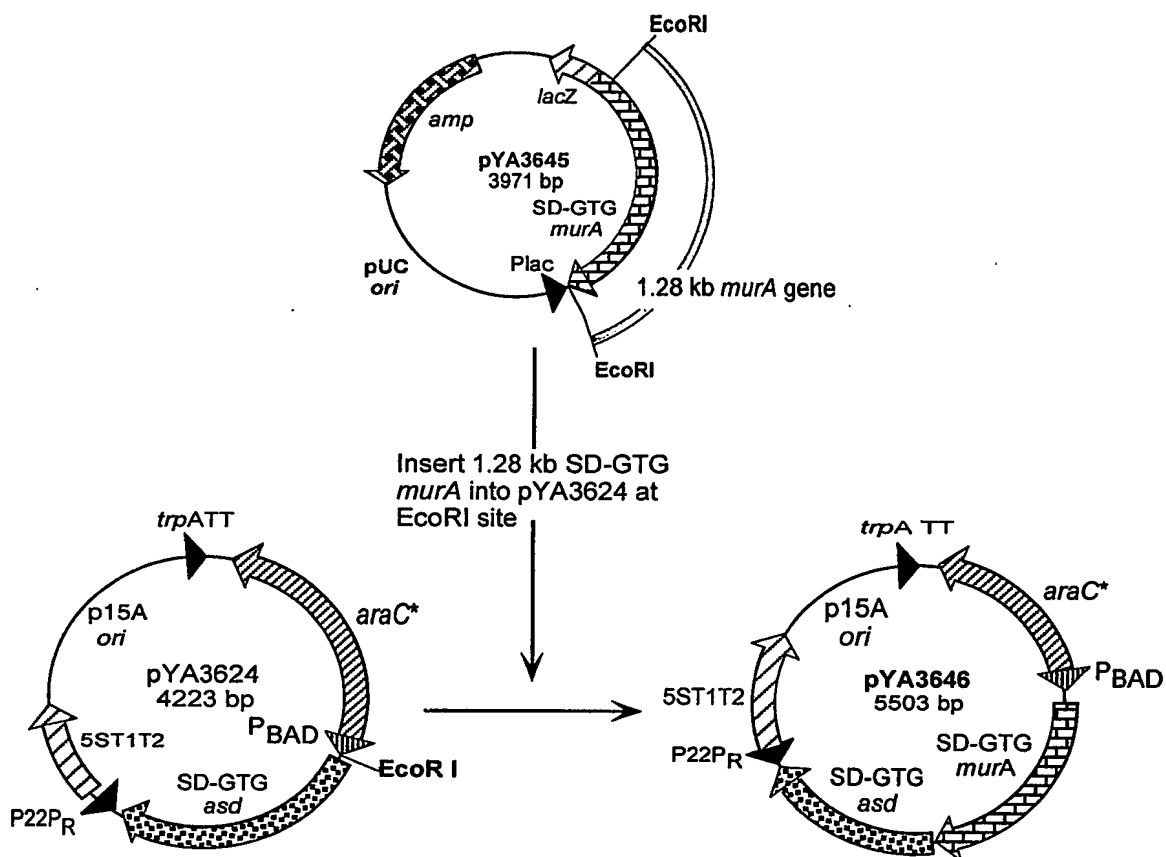


FIGURE 31. (continued)

## G. Construction of pYA3646.





**FIGURE 32. Cloning of *araC*P<sub>BAD</sub> from *E.coli* K-12 to achieve tighter regulation and a lower level of transcription in the absence of arabinose than is achievable with the existing *araC*P<sub>BAD</sub> system from *E.coli* B/r**

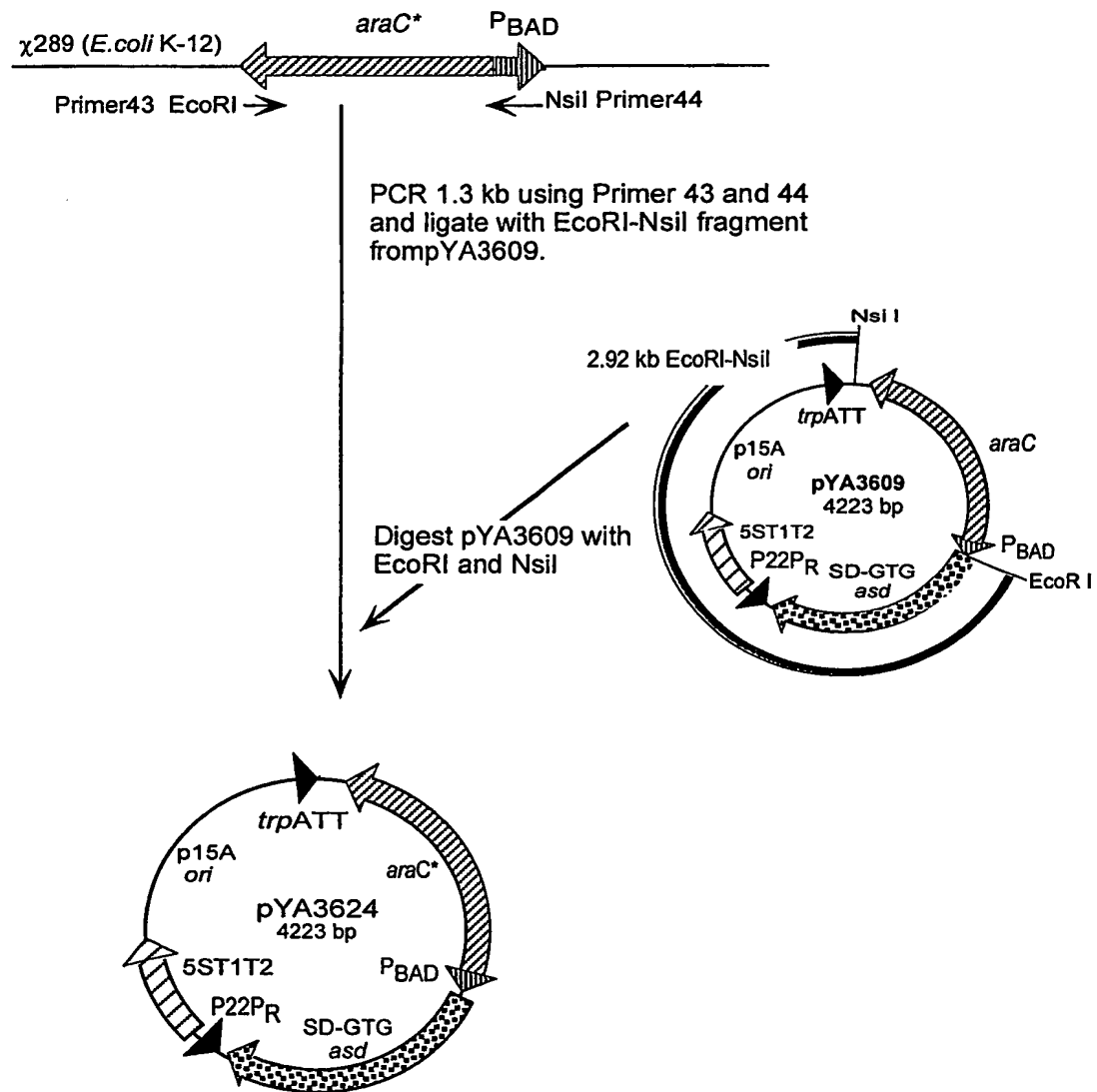


FIGURE 33. DNA nucleotide sequence of *araC*\*P<sub>BAD</sub> region from  $\chi$ 289 in pYA3624 and amino acid sequence of *AraC*\* protein

5' -GAATTCGCTA GCCCAAAA ACGGATATGG AGAAACAGTA GAGAGTTGCG ATAAAAAGCG TCAGGTAGGA TCCGCTAATC TTATGGATAA  
 EcoRI NheI -10 -35  
 AAATGCTATG GCATAGCAAA GTGTGACGCC GTGCAAAATA TCAATGTGGA CTTTCTGCC GTGATTATAG ACATTTTGT TACGCGTTT  
 araO<sub>1</sub>L  
 TGTGATGGCT TTGGTCCGC TTGTGTACAG AATGCTTTTA ATAAGCGGGG TTACCGGTTG GGTAGCGAG AAGAGCCAGT AAAAGACGCA  
 -10 +1 *araC*\* mRNA →  
 GTGACGGCAA TGTCTGATGC AATATGGACA ATTGGTTTCT TCTCTGAATG GTGGGAGTAT GAAAAGT -  
 araO<sub>2</sub>  
*araC*\* start codon  
 ATG GCT GAA GCG CAA AAT GAT CCC CTG CTG CCG GGA TAC TCG TTT AAC GCC CAT CTG GTG GCG GGT TTA ACG CCG ATT GAG GCC AAC GGT  
 M A E A Q N D P L L P L P G Y S F N A H L V A G L T P I E A N G  
 TAT CTC GAT TTT ATC GAC CGA CCG CTG GGA ATG AAA GGT TAT ATT CTC AAT ACC ATT CGC GGT CAG GGG GTG GTG AAA AAT CAG  
 Y L D F I D R P L G M K G Y I L N L T I R G Q G V V K N Q  
 1  
 GGA CGA GAA TTT GTC TGC CGA CCG GGT GAT ATT TTG CTG TTC CCG CCA GGA GAG ATT CAT CAC TAC GGT CGT CAT CCG GAG GCT CGC GAA  
 G R E F V C R P G D I L L F P P G E I H H Y G R H P E A R E  
 TGG TAT CAC CAG TGG GTT TAC TTT CGT CCG GCG GCC TAC TGG CAT GAA TGG CTT AAC TGG CCG TCA ATA TTT GCC AAT ACG GGT TTC TTT  
 W Y .H Q W V Y F R P R A Y W H E W L N W P S I F A N T G F F  
 CGC CCG GAT GAA GCG CAC CAG CCG CAT TTC AGC GAC CTG TTT GGG CAA ATC ATT AAC GCC GGG CAA GGG GAA GGG CGC TAT TCG GAG CTG  
 R P D E A H Q P H F S D L F G Q I I N A G Q G E G R Y S E L  
 CTG GCG ATA AAT CTG CTT GAG CAA TTG TTA CTG CCG GCG ATG GAA GCG ATT AAC GAG TCG CTC CAT CCA CCG ATG GAT AAT CCG GTA CGC  
 L A I N L L E Q L L L R R M E A I N E S L H P P M D N R V R  
 GAG GCT TGT CAG TAC ATC AGC GAT CAC CTG GCA GAC AGC AAT TTT GAT ATC GCC AGC GTC GCA CAG CAT GTT TGC TTG TCG CCG TCG CGT  
 E A C Q Y I S D H L A D S N F D I A S V A Q H V C L S P S R  
 CTG TCA CAT CTT TTC CGC CAG CAG TTA GGG ATT AGC GTC TTA AGC TGG CCG GAG GAC CAA CGC ATT AGT CAG GCG AAG CTG CTT TTG AGC  
 L S H L F R Q Q L G I S V L S W R E D Q R I S Q A K L L L S  
 ACT ACC CGG ATG CCT ATC GCC ACC GTC GGT CGC AAT GTT GGT TTT GAC GAT CAA CTC TAT TTC TCG CGA GTA TTT AAA AAA TGC ACC GGG  
 T T R M P I A T V G R N V G F D D Q L Y F S R V F K K C T G  
 GCC AGC CCG AGC GAG TTT CGT GCC GGT TGT GAA GAA AAA GTG AAT GAT GTA GCC GTC AAG TTG TCA TAA TTGGTAACGA ATCAGACAAT  
 A S P S E F R A G C E E K V N D V A V K L S \*  
 stop codon  
 TGACGGCTTG ACGAGTAGC ATAGGTTTG CAGAATCCCT GCTTCGTCCA TTGACAGGC ACAATTATGCAT -3'  
 NsiI

**FIGURE 34. DNA nucleotide sequences alignment of the *E.coli* K-12 *araCP<sub>BAD</sub>* region and the *E.coli* B/r *araCP<sub>BAD</sub>* region**

<i>E. coli</i> K-12	1	CCAAAAAACGGGTATGGAGAAACAGTAGAGAGTTGGCATAAAAAAGCGTCAGGTAGGATCCGCTAACTTATGGATAAAAAATGCTATGGCATAGCAAACT	100
<i>E. coli</i> B/r	1	CCAAAAAACGGGTATGGAGAAACAGTAGAGAGTTGGCATAAAAAAGCGTCAGGTAGGATCCGCTAACTTATGGATAAAAAATGCTATGGCATAGCAAACT	100
		• 20 • 40 • 60 • 80 • 100	
		• 120 • 140 • 160 • 180 • 200	
	101	GTGACGCGTGCAATAATCAATGTGGACTTTTCTGCGGTGATATAGACACTTTTGTACGGTTTTTGTTCATGGCTTTGGTCCCGCTTTGTTACAGAA	200
	101	GTGACGCGTGCAATAATCAATGTGGACTTTTCTGCGGTGATATAGACACTTTTGTTCATGGCTTTGGTCCCGCTTTGTTACAGAA	200
		• 120 • 140 • 160 • 180 • 200	
		• 220 • 240 • 260 • 280 • 300	
	201	TCCTTTTAATAGCGGGGTACCGGTTGGTTAGCGGAGAGAGCCAGTAAAGACGAGTACGGCAATGCTCATGCAATATGGACAATTGGTTCTTC	300
	201	TCCTTTTAATAGCGGGGTACCGGTTGGTTAGCGGAGAGAGCCAGTAAAGACGAGTACGGCAATGCTCATGCAATATGGACAATTGGTTCTTC	300
		• 220 • 240 • 260 • 280 • 300	
		• 320 • 340 • 360 • 380 • 400	
	301	TCTGAATGGTGGGAGTATGAAAAGTATGGCTGAAGCGCAAATATGATCCCTGCTGCGGGATACCTCGTTAAAGCCCATCTGGTGGCGGGTTTAACGCGG	400
	301	TCTGAATGGCGGAGTATGAAAAGTATGGCTGAAGCGCAAATATGATCCCTGCTGCGGGATACCTCGTTAAAGCCCATCTGGTGGCGGGTTTAACGCGG	400
		• 320 • 340 • 360 • 380 • 400	
		• 420 • 440 • 460 • 480 • 500	
	401	ATTGAGGCCAACGGTTATCTCGATTTTTTATCGAACCGCTGGGAATGAAAGTTATTTCTCAATCTCACCATTTCGGGGTCAGGGGGTGGTGAAAA	500
	401	ATTGAGGCCAACGGTTATCTCGATTTTTTATCGAACCGCTGGGAATGAAAGTTATTTCTCAATCTCACCATTTCGGGGTCAGGGGGTGGTGAAAA	500
		• 420 • 440 • 460 • 480 • 500	
		• 520 • 540 • 560 • 580 • 600	
	501	ATCAGGGACGAGAAATTGTCTGCCACCGGGTGATATTTTGTGTTCCCGCCAGAGAGATTTCATCACTACGGTCGTCTATCCCGAGGCTCCGCAATGGTA	600
	501	ATCAGGGACGAGAAATTGTCTGCCACCGGGTGATATTTTGTGTTCCCGCCAGAGAGATTTCATCACTACGGTCGTCTATCCCGAGGCTCCGCAATGGTA	600
		• 520 • 540 • 560 • 580 • 600	

FIGURE 34. (continued)

*E.coli* K-12 601 TCACAGTGGGTTTACTTTTCGTCGCGCGCCTACTGGCAGTAAGTGGCTTAACCTGSCCGCTCAATATTGGCAATACGAGTTCTTTTCGCGCGGATGAAGCG 700  
|||||  
601 TCACAGTGGGTTTACTTTTCGTCGCGCGCCTACTGGCAGTAAGTGGCTTAACCTGSCCGCTCAATATTGGCAATACGAGTTCTTTTCGCGCGGATGAAGCG 700  
620 640 660 680 700  
720 740 760 780 800  
701 CACCAGCGGCATTTTCAGCGACCTGTTTGGGCAATCAATTACGCGCGGCAAGGGAGGGCGCTATTCCGAGCTGCTGGCGATAAATCTGCTTGAGCAAT 800  
|||||  
701 CACCAGCGGCATTTTCAGCGACCTGTTTGGGCAATCAATTACGCGCGGCAAGGGAGGGCGCTATTCCGAGCTGCTGGCGATAAATCTGCTTGAGCAAT 800  
720 740 760 780 800  
801 TGTTACTGCGGGCATGGAAGCGATTAAACGAGTCGCTCCATCCACGATGGATAATCCGGTACGCGAGGCTTGTCTAGTACATCAGCCGATCACCTGGCAGA 900  
|||||  
801 TGTTACTGCGGGCATGGAAGCGATTAAACGAGTCGCTCCATCCACGATGGATAATCCGGTACGCGAGGCTTGTCTAGTACATCAGCCGATCACCTGGCAGA 900  
820 840 860 880 900  
920 940 960 980 1000  
901 CAGCAATTTGATATCGCCAGCGTCCGACAGCATGTTTGTCTGTCGCGCTCGCTCTGTCAATCTTTCCGCGCAGCTTAGGGATTAGCGTCTTAAGC 1000  
|||||  
901 CAGCAATTTGATATCGCCAGCGTCCGACAGCATGTTTGTCTGTCGCGCTCGCTCTGTCAATCTTTCCGCGCAGCTTAGGGATTAGCGTCTTAAGC 1000  
920 940 960 980 1000  
1001 TGCGCGGAGGACCAACGCAATAGTCAGGCGAAGCTGCTTTTGAGCATACCGCGATGCCCTATCGCCACCGTCGGTCGCAATGTTGGTTTTCAGCATCAAC 1100  
|||||  
1001 TGCGCGGAGGACCAACGCAATAGTCAGGCGAAGCTGCTTTTGAGCATACCGCGATGCCCTATCGCCACCGTCGGTCGCAATGTTGGTTTTCAGCATCAAC 1100  
1020 1040 1060 1080 1100  
1101 TCTATTCTCGCGGTATTTAAATAATGACCGGGCCAGCCCGAGGAGTTTCGTGCGCGGTGTAAGAAAGTAAGTATGAGCCGTCAGCTTGC 1200  
|||||  
1101 TCTATTCTCGCGGTATTTAAATAATGACCGGGCCAGCCCGAGGAGTTTCGTGCGCGGTGTAAGAAAGTAAGTATGAGCCGTCAGCTTGC 1200  
1120 1140 1160 1180 1200  
1201 ATAAATGGTAAAGTAATGACGCTTTCAGCGAGTAGCATAGGTTTGCAGAAATCCCTGCTTCGTCATTTCAGAGGCACATTATGCA 1300  
|||||  
1201 ATAAATGGTAAAGTAATGACGCTTTCAGCGAGTAGCATAGGTTTGCAGAAATCCCTGCTTCGTCATTTCAGAGGCACATTATGCA 1300  
1220 1240 1260 1280 1300

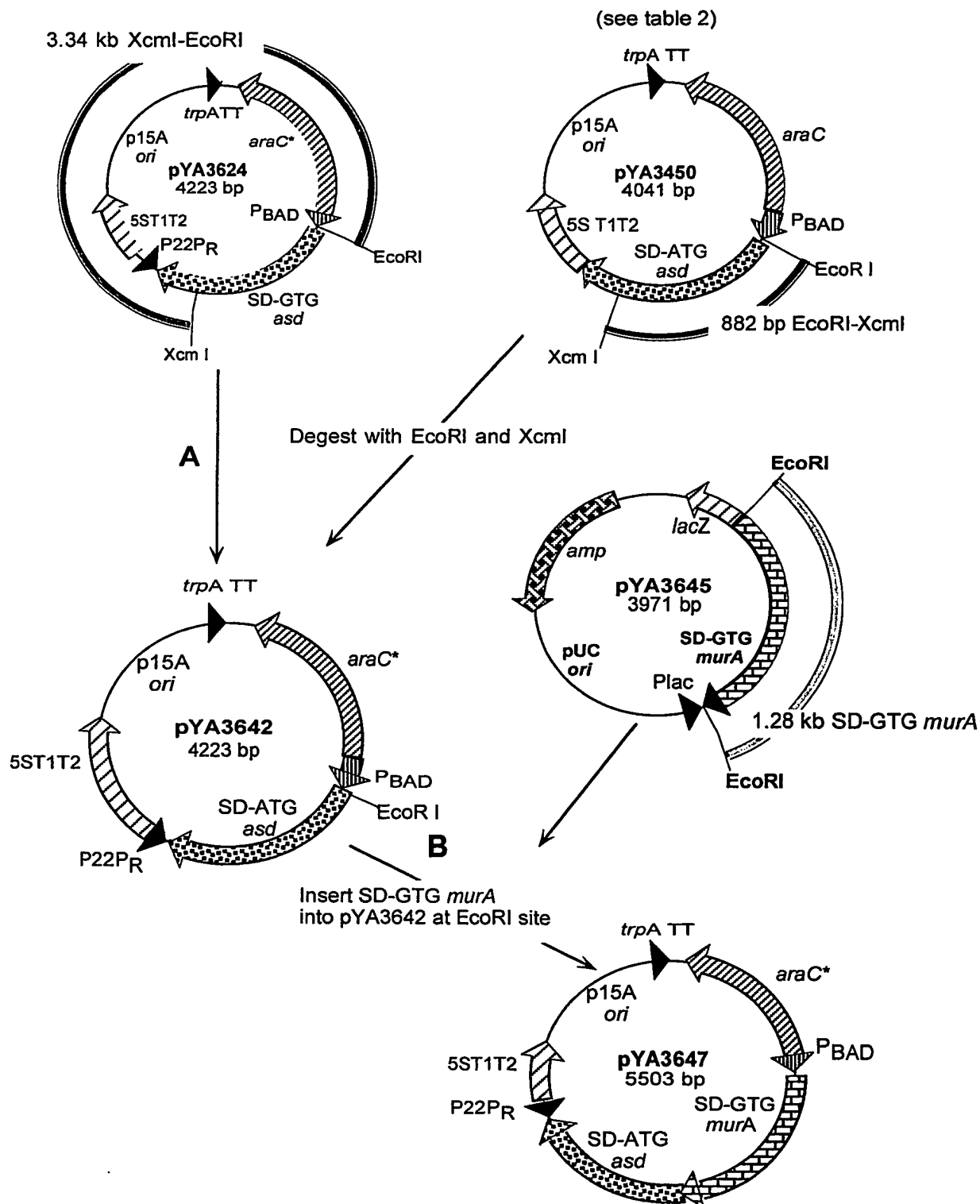
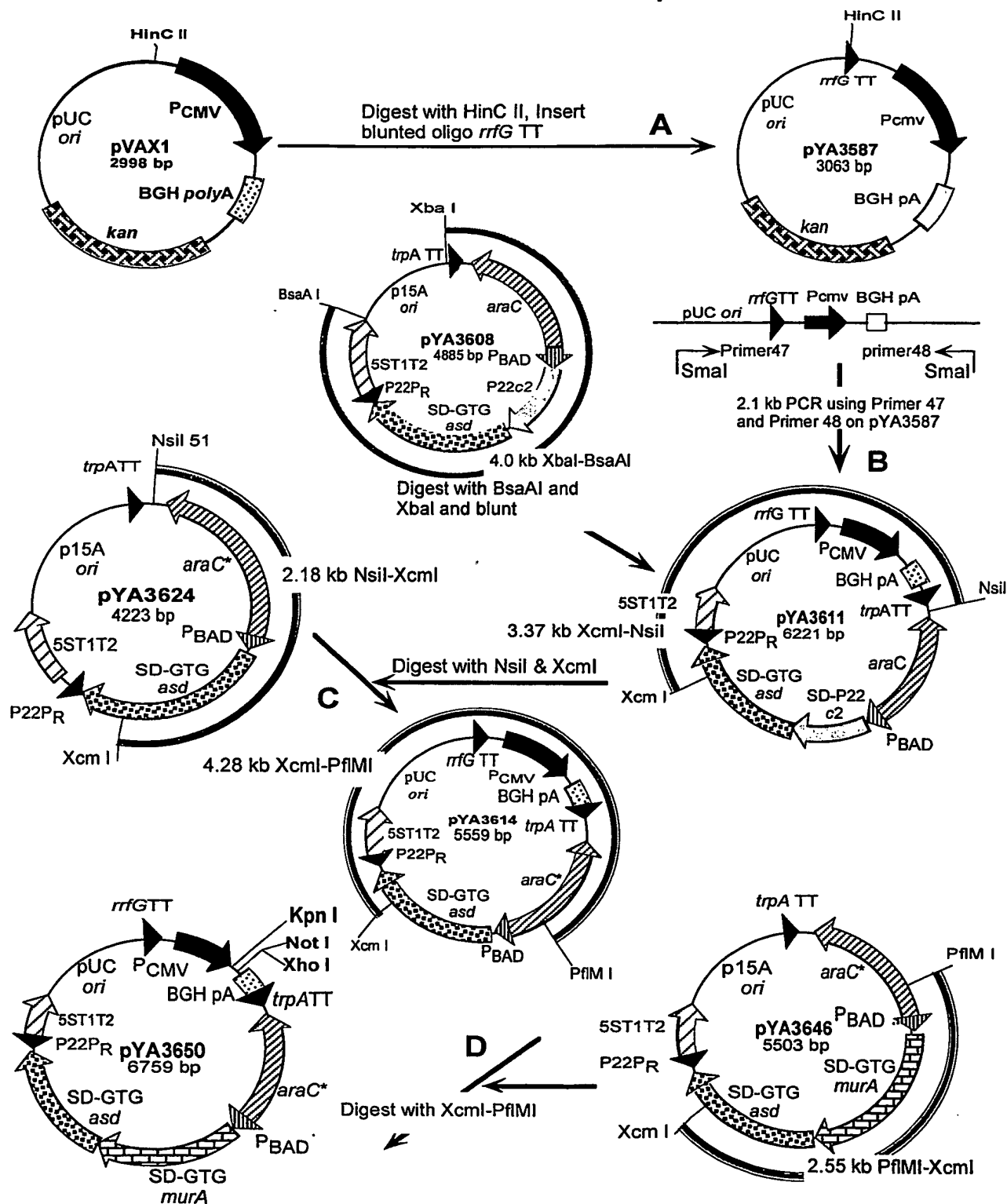
**FIGURE 35. Construction of the regulatable lysis system vector pYA3647**

FIGURE 36. Construction of the DNA vaccine vector pYA3650:



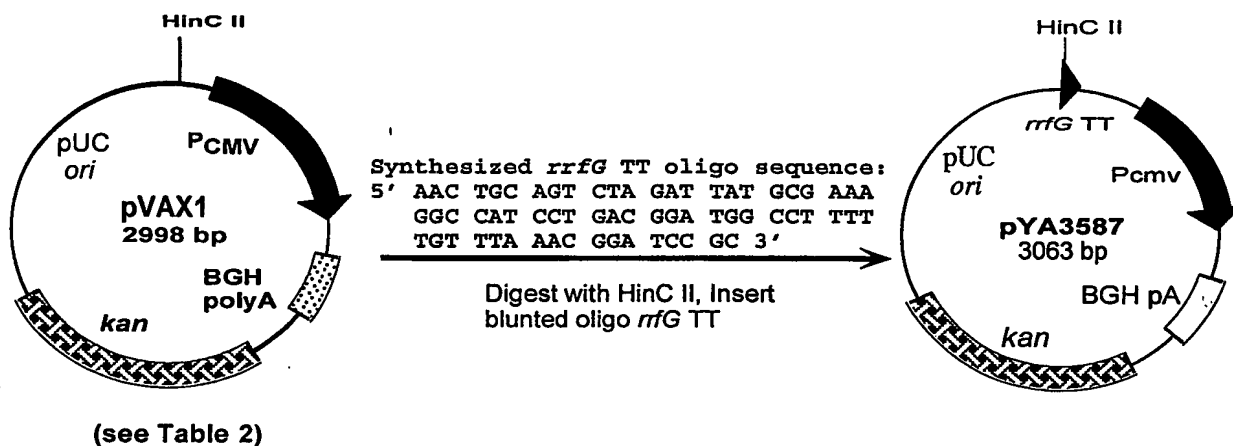
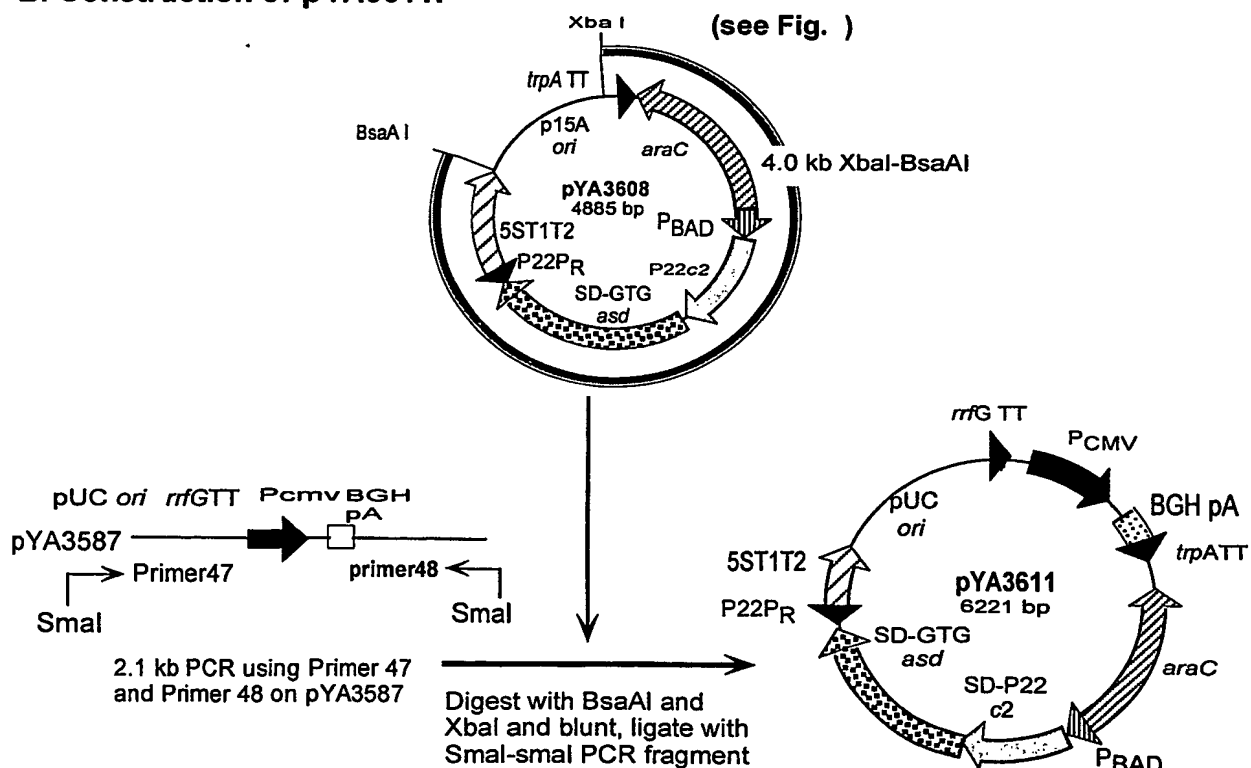
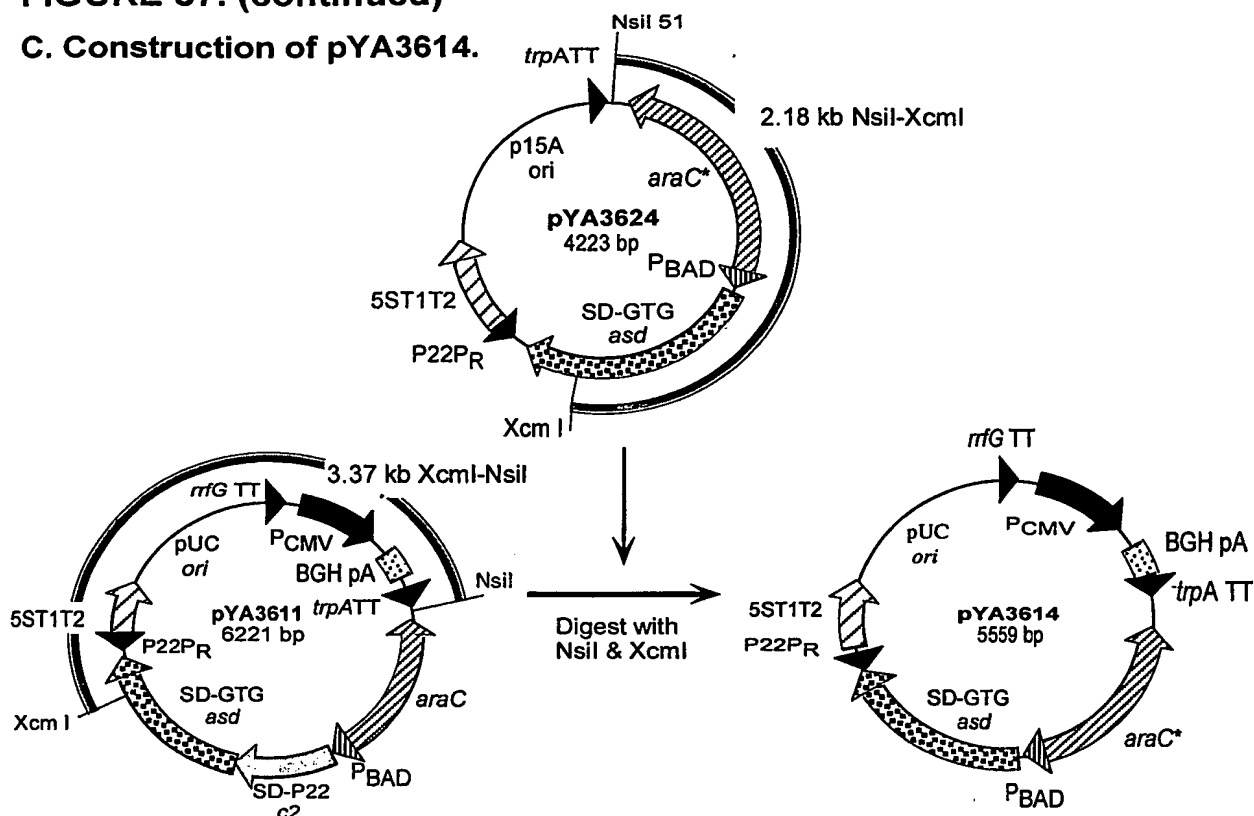
**FIGURE 37. Steps in the construction of pYA3650****A. Construction of pYA3587.****B. Construction of pYA3611.**

FIGURE 37. (continued)

## C. Construction of pYA3614.



## D. Construction of pYA3650.

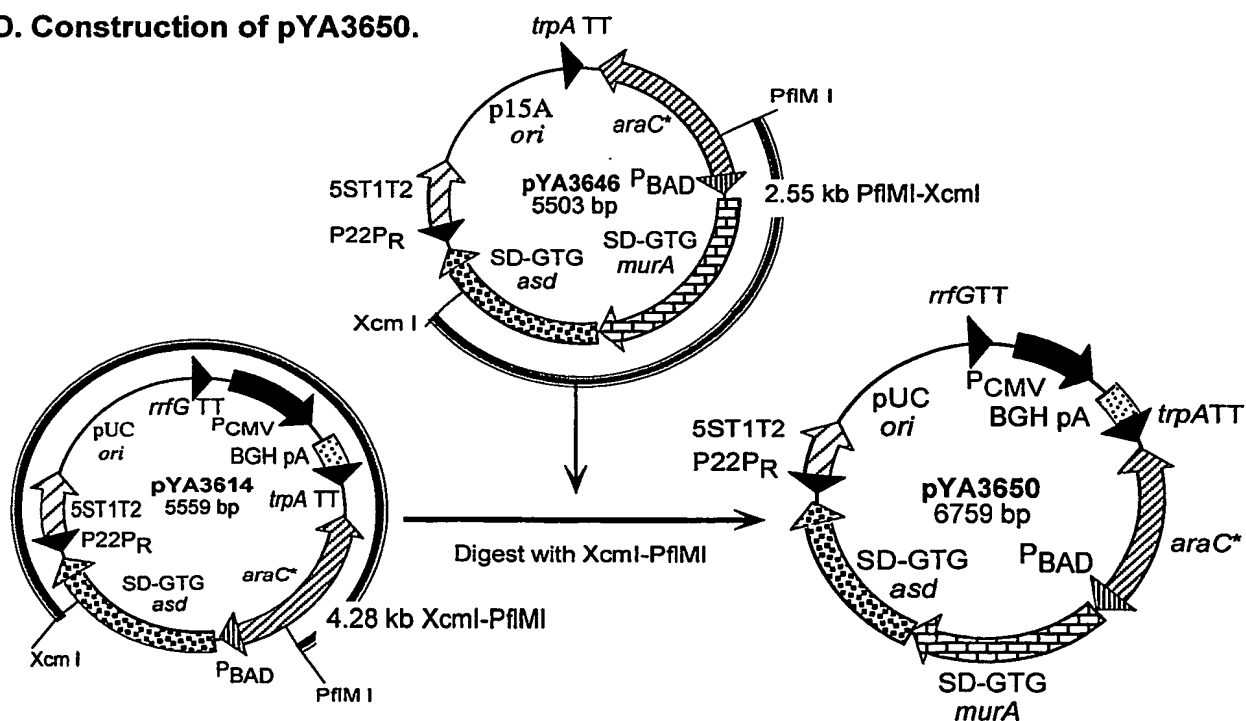




FIGURE 38. DNA sequence of DNA vaccine vector pYA3650 A. (1-3300 bp)

```

1  GACTCTTCGC GATGTACGG CCAGATATAC GCGTTAACTG CAGTCTAGAT TATGCCAAG GCCATCTGA CGGATGCGCT TTTTGTAA ACGGATCCGC 100
101 GACATTGATT ATTGACTAGT TATTATAGT GGGGTATTA GTTCAGCC CATATAGGA GTTCGCGT ACATACTTA CGGTAATGG 200
201 CCGCGCTGGC TGACCGCCCA AGACCCCGG CCCATTGAGC TCAATTAAGA CGTATGTTCC CATATGTAAC CCAATAGGGA CTTTCCATTG ACGTCAATGG 300
301 GTGGACTATT TACGGTAAC TGCCCACTTG GCAGTACATC AAGTGTATCA TATGCCAAGT AGCCCCCTA TTGACGTCAA TGACGGTAAA TGGCCGCGCT 400
401 GGCATTATGC CCAGTACATG ACCTTATGG ACTTCTCTAC TTGGCAGTAC ATCTAGTAT TAGTCATGC TATTACCATG GTGATCGGT TTTGGCAGTA 500
501 CATCAATGGG CGTGGATPAG GGTTTGACTC ACGGGGATTT CCAAGTCTCC ACCCATGTA CGTCAATGG AGTTTGT TTTTGGCACAAA TCAACGGGAC 600
601 TTTCCTCAAT GTCGTAAACA CTCGGGCCCA TTGACGCAAA TTGACGGTAG GCGGTAGCG TGGGAGTCT ATATAAGCAG AGCTCTCTGG CTAACGTAGAG 700
701 AACCCACTGC TTACTGGCTT ATCGAAATTA ATACGACTCA CTATAGGGAG ACCCAAGCTG GCTAGCGTTT AAACCTTAAGC TTGGTACCGA GCTCGGATCC 800
801 ACTAGTCCAG TGTGGTGGAA TTCTGCAGAT ATCCAGACA GTGGCGGCGG CTCGAGTCTA GAGGCGCGT TTAACCCCGG TGATCAGCCT CGACTGTGCC 900
901 TTCTAGTTGC CAGCCATCTG TGTGTTGCC GTTCTGCTCT GTGCTATCT GTGGGTGGG GTGGGTGGG GACGACAGC AAGGGGAGG ATTGGGAAGA CAATAGCAGG CATGCTGGGG 1100
1001 GCATCGCATT GTCTGAGTAG GTGCTATCT GTTCTGAGT TCTATGGCT TCTATGGCT ACAGCAAGC AACCGAATT GGCAGTGA TTTTATCTCT TCNAATGTAG 1200
1101 CACCTGAAGT CAGCCCCATA CGATATAAGT TGTGTTGAGG TCTAGCCCGG CTAATGAGCG GCGTATGTA TTCCCGGATG TTCCCGGATG CATATGTGC 1300
1201 CTGTCAATG GACGAGCAG GGAITCTGCA AACCTTATGC TACTCGTCA AGCCGTCAT TGCTGATCT GTTACCAAT ATGACAACTT GACGCTACA 1400
1301 TCATTCACTT TTTCTTCACA ACCGGCAGCA AACTCGTCTG GGTGGCCCC GGTGCATTTT TTAATPACT CCGAGAAATA GAGTTGATCG TCAAAACCAA 1500
1401 CATTGCGACC GACGGTGGG ATAGGCATCC GGTGATGCT CAAGAAGC TTGCGCTGAC TAATGCGTTG GTCTCGCGC CAGCTTAAGA CAGTAAATCC 1600
1501 TAACTGCTGG CGGAAAGAT GTGACAGAG CGACGGCGAC AAGCAACAT GCTGTGCGAC GCTGGCAATA TCAAAATGTC TGTCTGCCAG GTGATCGCTG 1700
1601 ATGTACTGAC AAGCCTCGCG TACCGATTA TCCATCGGTG GATGGAGCA CTCGTTAATC GCTTCCATGC GCCGAGTAA CAATTGCTCA AGCAGATTTA 1800
1701 TCGCCAGCAG CTCGGAATAG CGCCCTTCCC CTTGCCCCGG GTTAATGATT TGCCCAACA GGTGCTGAA ATGCGGCTGG TGCGCTTCAT CCGGGCGAAA 1900
1801 GAAACCCGTA TTGGCAATA TTGACGGCCA GTTAAGCCAT TCATGCCAGT AGGCGGCGG ACRAAGTAA ACCCACTGGT GATACCAATC GCGAGCTCC 2000
1901 GGATGACGAC CGTAGTGATG AATCTCTCT GCGGGGAACA GCAAAATATC ACCCGTCCG CAGACAAAT CTOGTCCCTG ATTTTTCACC ACCCCTGAC 2100
2001 CGCGAATGGT GAGATTGAGA ATATAACCTT TCATTCCCAG CCGTCGGTGC ATAAAAAT CGAGATAACC GTTGGCCTCA ATCGGCGTTA AACCGGCCAC 2200
2101 CAGATGGCGG TTAACGAGT ATCCCGGCG CAGGGATCA TTTTGGGCTT CAGCCATCTC TTTCATPACT CCACCATCA GAGAGAAAC CAATTGTCCA 2300
2201 TATTGATCA GACATTGCCG TCATGCGTC TTTTACTGGC TTGCTCTATA TCACGCGAGA AAAGTCCACA TTGATATTTT GCACGGCGT ACACTTTGGT ATGCCATAGC 2400
2301 CCAAGCCAT GACAAAACG CGTAACAAA GTGCTCTATC CTGAGCTTT TTATGCAAC TCTCTACTGT TTCTCATAC CCGTTTTTTT GGGCTAGCGA ATTCTGAGAA 2500
2401 ATTTTATACC ATAAGATTAG CGGATCCTAC CTGAGCTTT TTATGCAAC TCTCTACTGT TTCTCATAC CCGTTTTTTT GGGCTAGCGA ATTCTGAGAA 2600
2501 CAACTAAGT GGATAAATTT GGTGTTCCAG GGCACACGAA GCTCCAGGGC GAAGTCAAA TTTCCGGCGC TAAATGCT GCTCTGCCCTA TCCTTTTGGC 2700
2601 CGCACTACTG GCGGAAGAC CCGTAGAGAT CCAGAAGCTC CCGAACTGA AAGAGTCCA TACATCAATG AAGTGTCTAA GCCAGTGGG TGCGAAAGTA 2800
2701 GAACTGAATG GTTCTGTGCA TATTGATGCC CCGGAGCTTA ATGTATCTG CGCACTTAC GATCTGGTTA AAACCTGCG TGCTTCTATC TGCGCGCTGG 2900
2801 GGCCTGCTGT AGCGCGCTTT GGTCAAGGGC AAGTTTCACT ACCTGGCGGT GTGACATCG GTGCGGCTC TCGTGAATCTA CACATTTCTG GCTCGGAACA 3000
2901 ATTAGCGCG ACCATCAAC TGAAGAAG TTACGTTAAA GCTTCGCTG ATGTCGTTT GAAAGTGA CATATCGTGA TGGATAAAGT CAGCGTTGGC 3100
3001 GCAACGGTGA CCATCATGTG TGCTGCAAC GCACCAAGT TATTGAAAC GCAGCGCGT AACCGGAGT CGTGATACC GCGAACTTCC 3200
3101 TGATTAGCT GGTGCGAAA ATTAGCGGT ATCGAAGGTG TGAAGGTTT AGGCGGCGGT GTCTATCGCG TTCTGCGCGA 3300

```

FIGURE 38. (continued) DNA sequence of DNA vaccine vector pYA3650 B. (3301- 6759bp)

3301 TCGTATCGAA ACCGGTACTT TCCTGGTGGC GCGGCGGATT TCCTGGCGGA AATTATCTG CCGTAACGGC CAGCCAGATA CTCTCGACGC CGTCTGGCG 3400  
 3401 AAACGTGGTG AGCTGGAGC GGACATCGAA GTCCGGCGAAG ACTGGATATG CTTGGATATG CATGCCAAAC GTCCGAGGC TGTAAACGTA CGTACCGCGC 3500  
 3501 CGCATCCGGC ATTCCCGACC GATATGCAGG CCAGTTTCAC GCTGTTGAAC CTGGTGGCAG AAGGACCGG GTTTATCACC GAAACGGTCT TTGAAAACCG 3600  
 3601 CTTTATGCAT GTGCCAGAC TGAGCCGTAT GCGCGCGCAC GCGGAAATCG AAAGCAATAC CGTTATTTGT CACGGTGTG AAAACCTTC TGGCGCACAG 3700  
 3701 GTTATGGCAA CCGATCTGC TGATCAGCA AGCTGTGTC CTTTGGTGGT TATTCGGAA GGGACGACG TGGTTCATCG TATTTATCAC ATCGATCGTG 3800  
 3801 GCTACGAACG CATTGAAGC AATGGTCGC CTTTGGTGGC TCTGTTCTCA TGCACGCAAT GGTAGAGGAG CGCGATTTCG ACGCTATTTC CCGTGTTCCT 3900  
 3901 GGTATTTATCG GCTGGCGCG AATGGTCGC CCCACCTTCG GGCACCTTC TGTGTTGATC GATGCGCTA AAGCGCTCG ATATCATCGT 4000  
 4001 CCCAGTTTG ACAGCGCGG ACACCTTCG GGCACCTTC CACCGGACG CTACAGGAG CTGTTGATCT GATGCGCTA AAGCGCTCG ATATCATCGT 4100  
 4101 GACCTGCCAG GCGGCGGATT ATACCAAGCA AATTTATCCA AAGCTGGCAG AAGCTGGCAG CACAGGTTAC TGGATTGATG GCTGCGCATG 4200  
 4201 AAAGATGATG CCATTTATAT TCTCGACCG GTCAACCCAG ACGTGAATAC CAGCGGCTCG AACATGGCG TGAAGACCTT TGTGGGCGGT AACTGTACCG 4300  
 4301 TTAGCCTGAT GTTGATGTCG CTGGGCGGTC TCTTTGCCA TAATCTCGTT GACTGGGTAT CCGTCCGCG CTTATCAGGC CCCTCCGCG GCGGCGCGCG 4400  
 4401 CCATATGCGC GAGCTGTAA CCCAGATGGG TCAGTTGTAT GGCATGTTCG CCGATGAAT GGCACGCGG TCCTCCGCA TTCCTTGAT TGAACGCAAA 4500  
 4501 GTTACGGCAT TGACCGCAG CCGCGAGCTG CCGTTGATG ACTTTGGGT ACCGCTGGG GGAAGCCTGA TCCCTGGAT TCCCTGGAT TGAACGCAAA 4600  
 4601 GCGAGAGCG CGAAGAGTG AAAGCCAGG CCGAACCAG CAAGTTCTC AATGCTGCT CTGTGATTC GGTGATTCG CCGTCCGCG 4700  
 4701 GCTCGGCTGT CACAGCCAG CGTTACCAT CAGCTGAAA AAGAGGAT CCAATCCGAC GGTGGAAGAA CTGCTGGCG CACATAATCC GTGGCGAAA 4800  
 4801 GTGTGCGCA ACGATCGTGA TATCACTATG CCGCAATTA CCGCGCGCG GTGACCCGC GTGACCTGA CCGCTGGTGG TCGTCTGCGT AAGCTGAACA 4900  
 4901 TGGGCGCAGA GTTCTGTGCG GCGTTTACCG TAGGCGACCA GTTGTATGG GCGCGCGCG ACTTAATTA TCTACTAAAG GAATCTTTAG TCAAGTTTAT TTAAGATGAC 5000  
 5001 CTGCACGATA CCGTGCATT GTACATAGC GTAGGATCT TCCATTATG AAGCATTTAT CAGGTTTAT CAGGTTTAT GTCTCATGAG CTTGGCTGTT TTGGCGGATG 5100  
 5101 TTAACATAGA ATACACAATT GATGGGTGAG TACAGATTGA ATCAGAACG AGAAGCGGTG TGATAAACA GTTTCCTGG GGTGCTGAG CCGGTGCTC CACCTGACCC 5200  
 5201 AGAAGAGATT TTACAGCCTGA TACAGATTGA AACGCGTAG CCGCGATGGT AGTGTGGGT CTCCCATGC GAGAGTAGG AACTGCCAG CATCAATAA AACGAAAGGC 5300  
 5301 CAGTCCGAA TCAGAGTGA AAGCGGTG TCGGTTTAT TCGGTTTAT TCGGTTAAG CAGAGGCGA TCCCTGACGGA CGGATTGAA CGTTGCGAAG 5400  
 5401 TCAGTCCGAA GACTGGGCTT TCGGTTTAT TCGGTTTAT TCGGTTAAG CAGAGGCGA TCCCTGACGGA CGGATTGAA CGGCTTTTTC GCGTTCTAC 5500  
 5501 CAACGGCCCG GAGGTGGCG GGCAGGACGC CCGCCATAA TATCCGCTCA TGAGACAATA ACCCTGATAA ATGCTTCAAT AATGGAAGAT CTTCCAACAT 5600  
 5601 AAACCTCTTT TGTATTATTT TCTAATACA TTCAATATG TATCCGCTCA TGAGACAATA ACCCTGATAA ATGCTTCAAT AATGGAAGAT CTTCCAACAT 5700  
 5701 CACAGGTAAA CAGAAACGTC GGTTCGATCG GGAATCTTT TCCCGACCG CCGGGGTG GCAAGCCGC AGGCGCTCA GTGCTTTTAG CCGGTGTCG 5800  
 5801 GCGAGCCCTG AACAGTCAC GGGATCGATC TGTGCGGTAT TTCAACCCG ATACAGGTG GCAAGCCGC AGGCGCTCA GTGCTTTTAG CCGGTGTCG 5900  
 5901 TTTTCTTAAA TACATTCAA TATGATFCCG CTCATGAGC AATAACCTG AATAACCTG AATAACCTG AATAACCTG AATAACCTG AATAACCTG 6000  
 6001 GATCTAGGT GAATCTCTT TTTGATAATC TCATGACCAA AATCCCTTAA CGTGATTTT CGTTCCACTG AGCTCAGAC CCGTAGAAA AGATCAAGG 6100  
 6101 ATCTCTTTGA GATCTTTTTC TTTGATAATC TCATGACCAA AATCCCTTAA CGTGATTTT CGTTCCACTG AGCTCAGAC CCGTAGAAA AGATCAAGG 6200  
 6201 ACTCTTTTTC CAGAGGTAA TGGCTTCAGC AGAGCGCAGA TACCAATATC TGTCTCTTA GTGTAGCCGT AGTTAGGCCA CCACTTCAAG AACTGTGATG 6300  
 6301 CACCGCTTAC ATACCTCTGT CTGCTAATCC TGTGATFCCG CTCATGAGC AATAACCTG AATAACCTG AATAACCTG AATAACCTG AATAACCTG 6400  
 6401 TGAAGAGCG CAGCGCTCG GGTGCTGTC ACACAGCCCA GGTATCCGAT GGTATCCGAT GGTATCCGAT GGTATCCGAT GGTATCCGAT GGTATCCGAT 6500  
 6501 TGAGAAAGCG CCACGCTTCC CAGAGGGAGA AAGCGGAGA GGTATCCGAT GGTATCCGAT GGTATCCGAT GGTATCCGAT GGTATCCGAT 6600  
 6601 ACGCTGATA TCTTTATAGT CTTGCTGGGT TTCCGCCACT CTGATCTGAT GGTATCCGAT GGTATCCGAT GGTATCCGAT GGTATCCGAT 6700  
 6701 CAGCAACCGC GCCTTTTATG GGTCTCTGG CTTTGTGCTC ACATGTTCT 6759

**FIGURE 39. Oligo nucleotide sequence of synthesized *rrfG* TT and multiple cloning site of pYA3650**

**Synthesized *rrfG* TT oligo sequence:**

5' AAC TGC AGT CTA GAT TAT GCG AAA GGC CAT CCT GAC  
GGA TGG CCT TTT TGT TTA AAC GGA TCC GC 3'

**Multiple Cloning Site of pYA3650:**

AATTAATACG ACTCACTATA GGGAGACCCA AGCTGGCTAG CGTTTAAACT  
T7 promoter/priming site

TAAGCTTGGT ACCGAGCTCG GATCCACTAG TCCAGTGTGG TGAATTCTG  
KpnI

CAGATATCCA GCACAGTGGC GGCCGCTCGA GTCTAGAGGG CCCGTTTAAA  
NotI XhoI Stop codon

CCCGCTGATC AGCCTCGACT GTGCCTTCTA GTTGCCAGCC ATCTGTTGTT

TGCCCCCTCCC CCGTGCCTTC CTTGACCCTG GAAGGTGCCA CTCCCCTGT

CCTTTCCTAA TAAAATGAGG AAATTGCATC  
BGH poly A signal

10/526365

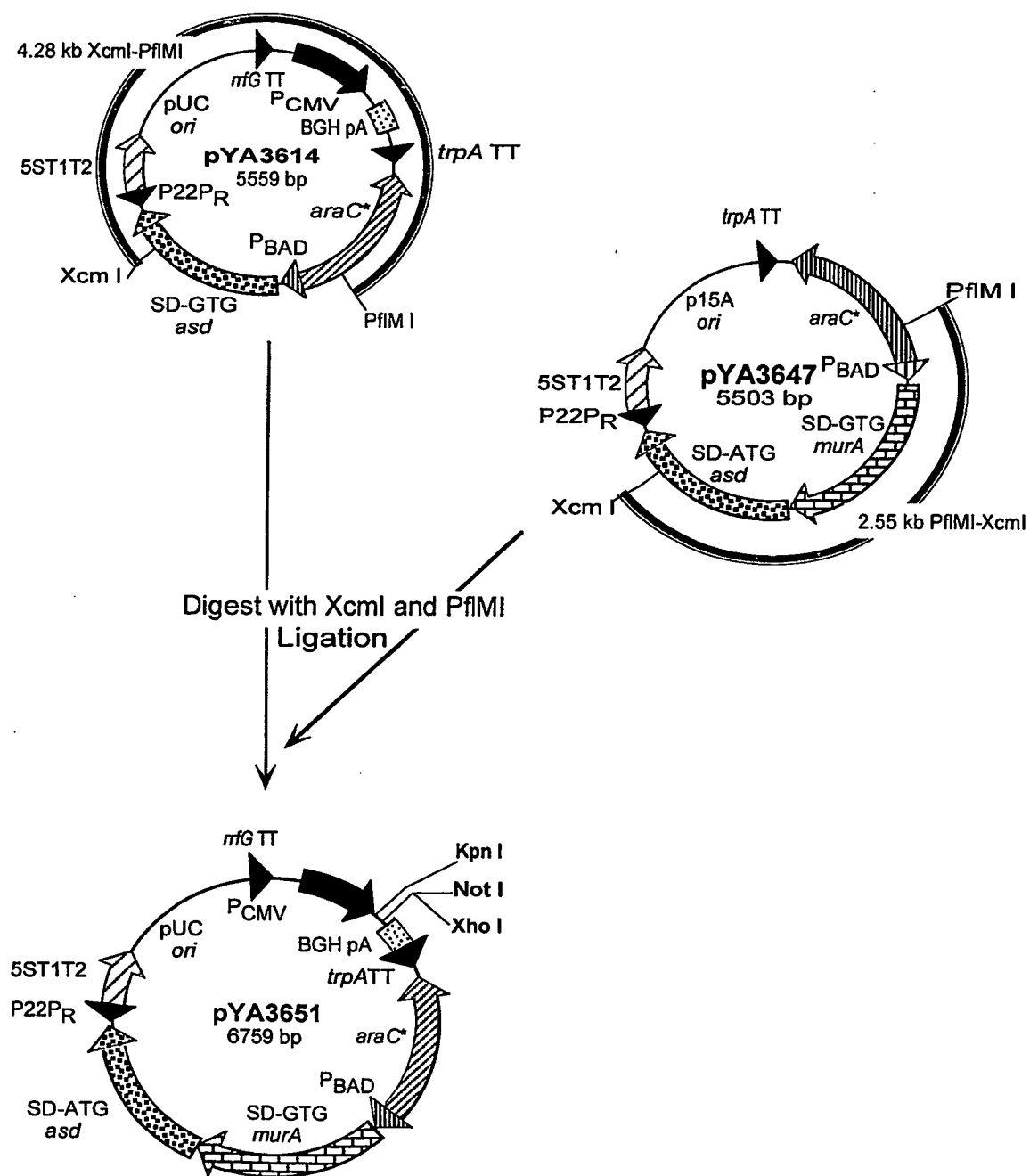
FIGURE 40. DNA and amino acid sequences of *GTG-murA* gene of pYA3650

1/1  
 GTG GAT AAA TTT CGT GTT CAG GGG CCA ACG AAG CTC CAG GGC GAA GTC ACA ATT TCC GGC GCT AAA AAT GCT GGT CTG CCT ATC CTT TTT  
 M D K F R V Q G P T K L Q G E V T I S G A K N A A L P I L F  
 91/31  
 GCC GCA CTA CTG GCG GAA GAA CCG GTA GAG ATC CAG AAC GTC CCG AAA CTG AAA GAC GTC GAT ACA TCA ATG AAG CTG CTA AGC CAG CTG  
 A A L L A E E P V E I Q N V P K L K D V D T S M K L L S Q L  
 181/61  
 GGT GCG AAA GTA GAA CGT AAT GGT TCT GTG CAT ATT GAT GCC CGC GAC GTT AAT GTA TTC TGC GCA CCT TAC GAT CTG GTT AAA ACC ATG  
 G A K V E R N G S V H I D A R D V N V F C A P Y D L V K T M  
 271/91  
 CGT GCT TCT ATC TGG GCG CTG GCG CCG CTG GCG GCG TTT GGT CAG GGG CAA GTT TCA CTA CCT GGC GGT TGT ACG ATC GGT GCG CGT  
 R A S I W A L G P L V A R F G Q G Q V S L P G C T I G A R  
 361/121  
 CCG GTT GAT CTA CAC ATT TCT GGC CTC GAA CAA TTA GGC GCG ACC ATC AAA CTG GAA GAA GGT TAC GTT AAA GCT TCC GTC GAT GGT CGT  
 P V D L H I S G L E Q L G A T I K L E E G Y V K A S V D G R  
 451/151  
 TTG AAA GGT GCA CAT ATC GTG ATG GAT AAA GTC AGC GTT GGC GCA ACG GTG ACC ATC ATG TGT GCT GCA ACC CTG GCG GAA GGC ACC ACG  
 L K G A H I V M D K V S V G A T V T I M C A A T L A E G T T  
 541/181  
 ATT ATT GAA AAC GCA GCG CGT GAA CCG GAA ATC GTC GAT ACC GCG AAC TTC CTG ATT ACG CTG GGT GCG AAA ATT AGC GGT CAG GGC ACC  
 I I E N A A R E P E I V D T A N F L I T L G A K I S G Q G T  
 631/211  
 GAT CGT ATC GTC ATC GAA GGT GTG GAA CGT TTA GGC GGC GGT GTC TAT CGC GTT CTG CCG GAT CGT ATC GAA ACC GGT ACT TTC CTG GTG  
 D R I V I E G V E R L G G G G V Y R V L P D R I E T G T F L V  
 721/241  
 GCG GCG CCG ATT TCT CCG GGC AAA ATT ATC TGC CGT AAC CCG CAG CCA GAT ACT CTC GAC GCC GTG CTG CCG AAA CTG CCG GAC GCT GGA  
 A A A I S R G K I I C R N A Q P D T L D A V L A K L R D A G  
 811/271  
 CCG GAC ATC GAA GTC GGC GAA GAC TGG ATT AGC CTG GAT ATG CAT GGC AAA CGT CCG AAG GCT GTT AAC GTA CGT ACC GCG CCG CAT CCG  
 A D I E V G E D W I S L L D M H G K R P K A V N V R T A P H P  
 901/301  
 GCA TTC CCG ACC GAT ATG CAG GCC CAG TTC ACG CTG TTG AAC CTG GCA GAA GGG ACC GGG TTT ATC ACC GAA ACG GTC TTT GAA AAC  
 A F P T D M Q A Q F T L L N L V A E G T G F I T E T V F E N  
 991/331  
 CGC TTT ATG CAT GTG CCA GAG CTG AGC CGT ATG GGC CCG CAC GCC GAA ATC GAA AGC AAT ACC GTT ATT TGT CAC GGT GTT GAA AAA CTT  
 R F M H V P E L S R M G A H A E I E S N T V I C H G V E K L  
 1081/361  
 TCT GGC GCA CAG GTT ATG GCA ACC GAT CTG GCT GCA TCA GCA AGC CTG GTG CTG GCT GGT ATT CGC GAA GGG ACG ACG GTG GTT GAT  
 S G A Q V M A T D L R A S A S L V L A G C I A E G T T V V D  
 1171/391  
 CGT ATT TAT CAC ATC GAT CGT GGC TAC GAA CGC ATT GAA GAC AAA CTG CGC GCT TTA GGT GCA AAT ATT GAG CGT GTG AAA GGC GAA TAA  
 R I Y H I D R G Y E R I E D K L R A L G A N I E R V K G E \*

**FIGURE 41. DNA and amino acid sequences of GTG-asd gene of pYA3650**

1/1  
 GTG AAA AAT GTT GGT TTT ATC GGC TGG CGC GGA ATG GTC GGC TCT GTT CTC ATG CAA CGC ATG GTA GAG GAG CGC GAT TTC GAC GCT ATT  
 M K N V G G F I G W R G C M V G S V L M Q R M V E E R D F D A I  
 91/31  
 CGC CCT GTT TTC TTT TCT ACC TCC CAG TTT GGA CAG GCG GCG CCC ACC TTC GGC GAC ACC TCC ACC GGC ACG CTA CAG GAC GCT TTT GAT  
 R P V F F S T S Q F G Q A A P T F G D T S T G T L Q D A F D  
 181/61  
 CTG GAT GCG CTA AAA GCG CTC GAT ATC ATC GTC ACC TGC CAG GGC GGC GAT TAT ACC AAC GAA ATT TAT CCA AAG CTG CGC GAA AGC GGA  
 L D A L K A L D I I V T C Q G G D Y T N E I Y P K L R E S G  
 271/91  
 TGG CAG GGT TAC TGG ATT GAT GCG GCT TCT ACG CTG CGC ATG AAA GAT GAT GCC ATT ATT ATT CTC GAC CGC GTC AAC CAG GAC GTG ATT  
 W Q G Y W I D A A S T L R M K D D A I I L D P V N Q D V I  
 361/121  
 ACC GAC GGC CTG AAC AAT GGC GTG AAG ACC TTT GTG GGC GGT AAC TGT ACC GTT AGC CTG ATG TTG ATG TCG CTG GGC GGT CTC TTT GCC  
 T D G L N N G V K T F V G G N C T V S L M L M S L G G L F A  
 451/151  
 CAT AAT CTC GTT GAC TGG GTA TCC GTC GCG ACC TAT CAG GCC GCC TCC GGC GGC GCG CAT ATG CGC GAG CTG TTA ACC CAG ATG  
 H N L V D W V S V A T Y Q A A S G G A R H M R E L L T Q M  
 541/181  
 GGT CAG TTG TAT GGC CAT GTC GCC GAT GAA CTG GCG ACG CCG TCT TCC GCA ATT CTT GAT ATT GAA CGC AAA GTT ACG GCA TTG ACC CGC  
 G Q L Y G H V A D E L A T P S S A I L D I E R K V T A L T R  
 631/211  
 AGC GGC GAG CTG CCG GTT GAT AAC TTT GGC GTA CCG CTG GCG GGA AGC CTG ATC CCC TGG ATC GAC AAA CAG CTC GAT AAC GGC CAG AGC  
 S G E L P V D N F G V P L A G S L I P W I D K Q L D N G Q S  
 721/241  
 CGC GAA GAG TGG AAA GGC CAG GCG GAA ACC AAC AAG ATT CTC AAT ACT GCC TCT GTG ATT CCG GTT GAT GGT TTG TGT GTG CGC GTC GGC  
 R E E W K G Q A E T N K I L N T A S V I P V D G L C V R V G  
 811/271  
 GCG CTG CCG TGT CAC AGC CAG GCG TTC ACC AAG CTG AAC GAG GTA TCC ATT CCG ACG GTG GAA GAA CTG CTG GCG GCA CAT AAT  
 A L R C H S Q A F T I K L K K E V S I P T V E E L L A A H N  
 901/301  
 CCG TGG GCG AAA GTG GTG CCG AAC GAT CGT GAT ATC ACT ATG CGC GAA TTA ACC CCG GCG GTG ACC GGC ACG TTG ACT ACG CCG GTT  
 P W A K V V P N D R D I T M R E L T P A A V T G T L T T P V  
 991/331  
 GGT CGT CTG CGT AAG CTG AAC ATG GCG CCA GAG TTC TTG TCG GCG TTT ACC GTA GGC GAC CAG TTG TTA TGG GGC GCC GCC GAG CCG CTG  
 G R L R K L N M G P E F L S A F T V G D Q L L W G A A E P L  
 1081/361  
 CGT CGA ATG CTG CGC CAG TTG GCG TAG  
 R R M L R Q L A \*

FIGURE 42. Construction of the DNA vaccine vector pYA3651



**FIGURE 43. DNA sequence of the DNA vaccine vector pYA3651 A. (1-3300 bp)**

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1  GACTCTTCGC GATGTACGGG CCAGATATAC GCGTTAACTG CAGTCTAGAT TATGGGAAAG GCCATCTCTG CCGATGGCCT TTTTGTTTAA ACGGATCCGC 100
101 GACATTGAT ATTGACTAGT TATTATATAGT AATCAATTAC GGGGTCAATTA GTTCATAGCC CATATATGGA GTTCGCGGTT ACNTAACTTA CGGTAAATGG 200
201 CCCGCCGTGG TGACCCGCCA ACGACCCCGG CCCATTGACG TCAATATATGA CGTATGTTC CATAGTAACG CCAATAGGGA CTTTCCATTG ACGTCAATGG 300
301 GTGGACTATT TACGGTAAAC CAGTACATG ACCTTATGGG ACTTTCCTAT TTGGCAGTAC ATCTAGCTAT TAGTCATCGC TAGTCATCGC TGACCGTAAA TGGCCCGCCT 400
401 GGCATTATGC CCAGTACATG ACCTTATGGG ACCTTATGGG ACTTTCCTAT TTGGCAGTAC ATCTAGCTAT TAGTCATCGC TAGTCATCGC TGACCGTAAA TGGCCCGCCT 500
501 CATCAATAGG GTGGATAGC GGTTCGATGC GGTTCGATGC GGTTCGATGC GGTTCGATGC GGTTCGATGC GGTTCGATGC GGTTCGATGC GGTTCGATGC 600
601 TTTCCTAAAT GTCGTAACAA CTCGCCGCCA TTGACGCAAA TGGGCGGTAG CCGTGTACGG GTGAGGTCT ATATAAGCAG AGCTCTCTGG CTAACCTAGAG 700
701 AACCCACTGC TTACTGGCTT ATCGAAATTA ATACGACTCA CTATAGGAG ACCCAAGCTG GCTAGCGTTT AAACCTAAGC TTGTATACCGA GCTCGGATCC 800
801 ACTAGTCCAG TGTGTGGAA TTCTGCAGAT ATCCAGCACA GTGGCGGCCG CTCGAGTCTA GAGGCGCCGT TTAACCCGCG TGATCAGCCT CGACTGTGCC 900
901 TTCTAGTTCG CAGCATCTG TTGTTTGCCG CTCCGCCGTG CCTTCTTGA CCTTGAAGG TGCCACTCCC ACTGTCTCTT CCTAATAAAA TGAGGAAATT 1000
1001 GCATCGCAT TTCTGAGTAG GTGTCAATCT ATCTTGGGG GTGGGTGGG ACAGCACAGC GACGACAGC AAGGCGGAG ATTGGGAAGA CAATAGCAGG CATGCTGGG 1100
1101 ATCGGTGGG CTCTATGGCT TCTATGGGCT GGTTCATAGG ACAGCAAGCG AACCAGATG GCCAGTAGA TTTCAGTGCA ATTTATCTCT TCAATGTAG 1200
1201 CACCTGAAGT CAGCCCATTA CGATATAAGT TGTTCGAAAG TCTAGCCCGC CTAATGAGCG GGTCTTTT TAATTCGCAA TTCCAGTGCA TTCCAGTGCA TTCCAGTGCA 1300
1301 CTGTCAATG GACGAGCAG GGAATCTGCA AACCTATGC TACTCGTCA AGCGTCAAT TGTCTGATC TTAATACTC GCGAGAAAT GAGTGTATCG TCAAAACCAA 1400
1401 TCATTCACT TTCTCTACA ACCGACAGA AACTCGCTCG GGTGCGGCC CAAAGCAGC TTCCGCTGAC TAATCGCTG GTCCTCGCG CAGCTTAA GCGTAAATCCC 1500
1501 TAATTGCTGG CAGGTGGCG ATAGCATCC CGACGCGAG ATAGCAACAT CTCTGCGAC GCTTGGCGA TCTTCCATGC GCGCAGTAA CAATGTCTCA AGCAGATTTA 1600
1601 TAATTGCTGG CAGGTGGCG ATAGCATCC CGACGCGAG ATAGCAACAT CTCTGCGAC GCTTGGCGA TCTTCCATGC GCGCAGTAA CAATGTCTCA AGCAGATTTA 1700
1701 ATGTACTGAC AAGCTCGCG TACCGGATTA TCCATCGGTG GATGAGCGA GCTTAAATG TTGCCAACA GGTCTGCTGA ATGCGGCTGG CCGGCGGAAA 1800
1801 TCGCCAGCAG CTCGCAATAG CGCCCTTCCC TTGACGGCCA GTTAAGCCAT TCATGCCAGT AAGCGCGCG ACGAAAGTAA ACCACTGGT GATACCATTC GCGAGCCTCC 1900
1901 GARACCCGTA TTGCAAAATA TTGACGGCCA GTTAAGCCAT TCATGCCAGT AAGCGCGCG ACGAAAGTAA ACCACTGGT GATACCATTC GCGAGCCTCC 2000
2001 GATGACGAC CGTAGTGATG AATCTCTCT GGCAGGGAACA GCATAATATC ACCCGTGG CAGACAAAT CTCTGCTCCG ATTTTTCACC ACCCTCTGAC 2100
2101 CGGATAGGT GAGATTGAGA ATATAACCTT TCATCCCAG CCGTCCGCTG ATAAAAAAT CAGATATACC GTTGGCTTCA ATGCGGCTTA AACCGCCAC 2200
2201 CAGATGGGG TTAAAGAGT ATCCGGCAG CAGGGATCA TTTTGGCGCT TTTTGGCGCT CAGCATATC CAGCATATC GTTATACATC GTTATACATC CAAATGTCCA 2300
2301 TATGTCATCA GACATGCGG TCACTGCGTC TTTTACTGCG TCTTCTCGCT AACCAACCG GTTAAACCG GTTAAACCG GTTAAACCG GTTAAACCG GTTAAACCG 2400
2401 CCAAGCCAT GACAAAACG CGTAAACAAA GTGTCTATAA TCAGGGCAGA AAGTCCACA TCTTACTGT TTCTCCATC CCGTTTTTTT GGGCTAGCGA ATCTGAGAA 2500
2501 ATTTTATCC ATAGATTAG CCGATCCTAC CTGACGCTTT TTATCGCAAC GAGTCCAGG GAAGTCCACA TCTTACTGT TTCTCCATC CCGTTTTTTT GGGCTAGCGA ATCTGAGAA 2600
2601 CAACTAAGT GATATAATT CGTGTTCAGG GGCACACGAA GCTCAACGAA CCGTCAAGG CCGTCAAGG CCGTCAAGG CCGTCAAGG CCGTCAAGG CCGTCAAGG 2700
2701 CGCATACTG GCGGAAGAAC TATTGATGCC CCGCAAGTTC CCGCAAGTTC CCGCAAGTTC CCGCAAGTTC CCGCAAGTTC CCGCAAGTTC CCGCAAGTTC 2800
2801 GAACGTAAT GTTCTGTGCA TATTGATGCC CCGCAAGTTC CCGCAAGTTC CCGCAAGTTC CCGCAAGTTC CCGCAAGTTC CCGCAAGTTC CCGCAAGTTC 2900
2901 AGCGCTGTG AGCGCTGTG AGCGCTGTG AGCGCTGTG AGCGCTGTG AGCGCTGTG AGCGCTGTG AGCGCTGTG AGCGCTGTG AGCGCTGTG 3000
3001 ATTAGCGCG ACCATCAAC TGAAGAAGG TTAGGTTAAA GCTTCCGTCG ATGCTCGTTT GAAAGTGC CAATATGCA TCGATTAAGT CAGCTGTGCG 3100
3101 GCAACGGTGA CCATCATGTG TGCTGCAACC CTGCGGGAAG GCACACCAT TATTGAAC GACGCGGTG AACCGGAAT CBTCTGATCC GCGAATCTCC 3200
3201 TGATTACGCT GGTGCGGAAA ATTAGCGGTC AGGCGACCGA TCGTATCTC ATCGAAGGTG TGAACGTTT AGCGCGCGGT GTCTATCGCG TTCTGCCCGA 3300

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**FIGURE 43. (continued) DNA sequence of the DNA vaccine vector pYA3651 B. (3301-6759bp)**

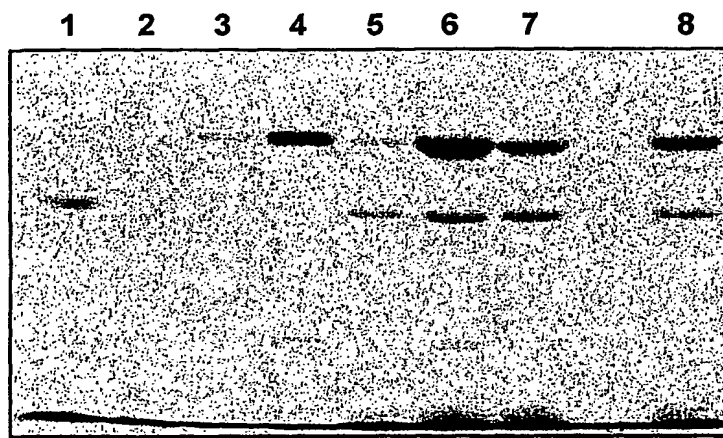
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 3501 CGCATCCGC ATTCGCCACC GATATGCAAG CCAGTTTAC GCTGTTGAAC CTGTGGCGAG AAGGACCGG GTTTATCACC GAAACGCTCT TTGAAAACCG 3600  
 3601 CTTTATGCA GTGCCAGAGC TGAGCCGTAT GGGCGCGCAC GCGCAATCG AAGCAATAC CGTTATTGT CACGGTGTG AAAAATTTT TGGCGCACAG 3700  
 3701 GTTATGGCAA CCGATCTGC TGCATCAGCA AGCTGTGTG TGGTGGGAA GGGACGACGG TGGTGTGATCG TATTTATCAC ATCGATCGTG 3800  
 3801 GCTACGAAC CATTAAGAC AAATGTCGG AATGTCGGC TCTGTTCTCA TGCAACGCT GGTAGAGGAG CGCATTTTC ACCTGTTTC TTTTCTACCT 3900  
 3901 GGTATTATCG ACGTGGCGG AATGTCGGC CTTTAGTTC GCGACCTTC CACCGCGAG CTACAGGAGC CTTTGTGATCT GGTAGGCTG ATATCATCGT 4000  
 4001 CCCAGTTGG ACAGGCGGG CCCACCTTC GCGACCTTC CACCGCGAG CTACAGGAGC CTTTGTGATCT GGTAGGCTG ATATCATCGT 4100  
 4101 GACCTGCCAG GGGCGGCAAT ATACCAACGA AATTTATCCA AAGCTGCGG AAGCGGATG GCAGGTTAC TGGATGATG GCTGCGCATG 4200  
 4201 AAGATGATG CCATTATTAT TCTCGACCG GTCAACCCAG ACGTGAATAC CGACGGCCTG AACATGGCG TGAAGACCTT TGTGGCGGT AACTGTACCG 4300  
 4301 TTAGCCTGAT GTTGATGTG CTGGGGGTC TCTTTGCCA TAATCTCGT GACTGGGTAT CCGTCGCGAC CTATCAGGC GCCTCCGGC GCGGCGCGCG 4400  
 4401 CCATATGCGC GAGCTGTAA CCCAGATGG TCAGTTGTAT GGCATGTTC GGCATGAACT GGCACGCGC TCTTCGCAA TTCTTGATAT TGAACGCAA 4500  
 4501 GTTACGGCAT TGACCGCAG CCGGAGCTG CCGTTGATA ACTTTGGCT ACCGCTGGG GGAAGCTGA TCCCTGGAT CGACAACAG CTCGATAACG 4600  
 4601 GCGAGAGCG CGAAGATGG AAGGCCAGG CCGGTTGATA ACTTTGGCT ACCGCTGGG GGAAGCTGA TCCCTGGAT CGACAACAG CTCGATAACG 4700  
 4701 GCTGCGCTGT CACAGCCAG CGTTCAACAT CAAGCTGAAA AAGAGGATAT CCATTCGAC GGTGGAAGAA CTGCTGGCG CACATAATCC GTGGCGAAA 4800  
 4801 GTGTGCGCA ACGATCGTGA TATCACTATG TAGGCGACCA GTTGTATGG GCGCGCGCG AGCGCTGG TCGTCTGCGT AAGCTGAACA 4900  
 4901 TGGGCGCAGA GTTCTGTGCG GCGTTTACCG GCGTTTACCG GCGTTTACCG GCGTTTACCG GCGTTTACCG GCGTTTACCG GCGTTTACCG 5000  
 5001 CTCACGATA CCGTCGACTT GTACATGAG GTAGTGTGAG CCGTGTGAG GTAGTGTGAG GTAGTGTGAG GTAGTGTGAG GTAGTGTGAG 5100  
 5101 TTAACATGA ATACCAATTT TTAACATGA ATACCAATTT TTAACATGA ATACCAATTT TTAACATGA ATACCAATTT TTAACATGA ATACCAATTT 5200  
 5201 AGAAGAGAT TTAACATGA ATACCAATTT TTAACATGA ATACCAATTT TTAACATGA ATACCAATTT TTAACATGA ATACCAATTT TTAACATGA ATACCAATTT 5300  
 5301 CATGCCGAC TCAGAGTGA AACGCCGTAG CCGCGATGT CCGCGATGT CCGCGATGT CCGCGATGT CCGCGATGT CCGCGATGT 5400  
 5401 TCACTCGAA GACTGGGCTT TCGTTTAT TCGTTTAT TCGTTTAT TCGTTTAT TCGTTTAT TCGTTTAT TCGTTTAT TCGTTTAT 5500  
 5501 CAACGCGCCG GAGGTGGCG GGCAGGACGC CCGCCATAAA CTGCCAGCA TCAAAATTAAG TAGGACAAAT CCGCGCGG CCGCGCGG 5600  
 5601 AAATCTTTT TGTATTATTT TCTAAATATG TATCCGCTCA TCCGCGTCA TCCGCGTCA TCCGCGTCA TCCGCGTCA TCCGCGTCA 5700  
 5701 CACAGGTAA CAGAACGTC GGTGCGATCG GGTGCGATCG GGTGCGATCG GGTGCGATCG GGTGCGATCG GGTGCGATCG GGTGCGATCG 5800  
 5801 GCGAGCCCTG AACAGTCAC GGTGCGATCG GGTGCGATCG GGTGCGATCG GGTGCGATCG GGTGCGATCG GGTGCGATCG GGTGCGATCG 5900  
 5901 TTTTCTTAA TACATTCAA TATGATATCG TATGATATCG TATGATATCG TATGATATCG TATGATATCG TATGATATCG TATGATATCG 6000  
 6001 GATCTAGGT GAAGATCTTT GAAGATCTTT GAAGATCTTT GAAGATCTTT GAAGATCTTT GAAGATCTTT GAAGATCTTT GAAGATCTTT 6100  
 6101 ATCTCTTGA GATCTCTTT GATCTCTTT GATCTCTTT GATCTCTTT GATCTCTTT GATCTCTTT GATCTCTTT GATCTCTTT 6200  
 6201 ACTCTTTTC CAGAGGTAAC TGGCTTCAGC AGAGCGCAGA TACCAATATC TACCAATATC TACCAATATC TACCAATATC TACCAATATC 6300  
 6301 CACCGCTTAC ATACTCTGT GGTCTAATCC TGTCTAATCC TGTCTAATCC TGTCTAATCC TGTCTAATCC TGTCTAATCC TGTCTAATCC 6400  
 6401 GATTAAGCG CACGGTCCG CTGTAACCGG GGGTTCGTG ACACAGCCCA AGTGGAGCG GGTGGAGCG GGTGGAGCG GGTGGAGCG 6500  
 6501 TGAGAAAGCG CACGCTTCC CGAAGGAGA AAGCGGAGA GGTATCCGT AAGCGGAGA GGTATCCGT AAGCGGAGA GGTATCCGT AAGCGGAGA 6600  
 6601 ACGCTGGTA TCTTTATAGT CTTGCTGGGT TTCGCGACCT CTGACTTGA CTGACTTGA CTGACTTGA CTGACTTGA CTGACTTGA 6700  
 6701 CAGCAACCGC GCCTTTTAT GGTTCCTGG CTTTGTGCTG CTTTGTGCTG CTTTGTGCTG CTTTGTGCTG CTTTGTGCTG 6759



**FIGURE 44. DNA and amino acid sequences of ATG-asd gene of pYA3651**

1/1  
 ATG AAA AAT GTT GGT TTT ATC GGC TGG CGC GGA ATG GTC GGC TCT GTT CTC ATG CAA CGC ATG GTA GAG GAG CGC GAT TTC GAC GCT ATT  
 M K N V G F I G W R G M V G S V L M Q R M V E E R D F D A I  
 31/21  
 91/31  
 CGC CCT GTT TTC TTT ACC TCC CAG TTT GGA CAG GCG GCG CCC ACC TTC GGC GAC ACC TCC ACC GGC ACG CTA CAG GAC GCT TTT GAT  
 R P V F F S T S Q F G Q A A P T F G D T S T G T L Q D A F D  
 151/51  
 181/61  
 CTG GAT GCG CTA AAA GCG CTC GAT ATC ATC GGC GGC GGC GAT TAT ACC AAC GAA ATT TAT CCA AAG CTG CGC GAA AGC GGA  
 L D A L K A L D I I V T C Q G G D Y T N E I Y P K L R E S G  
 241/81  
 271/91  
 TGG CAG GGT TAC TGG ATT GAT GCG GCT TCT ACG CTG CGC ATG AAA GAT GAT GGC ATT ATT ATT CTC GAC CCG GTC AAC CAG GAC GTG ATT  
 W Q G Y W I D A A S T L R M K D D A I I L D P V N Q D V I  
 331/111  
 361/121  
 ACC GAC GGC CTG AAC AAT GGC GTG AAG ACC TTT GTG GGC GGT AAC TGT ACC GTT AGC CTG ATG TTG ATG TCG CTG GGC GGT CTC TTT GCC  
 T D G L N N G V K T F V G G N C T V S L M L M S L G G L F A  
 421/141  
 451/151  
 CAT AAT CTC GTT GAC TGG GTA TCC GTC GCG ACC TAT CAG GCC GCC TCC GGC GGC GGC CAT ATG CGC GAG CTG TTA ACC CAG ATG  
 H N L V D W V S V A T Y Q A A S G G A R H M R E L L T Q M  
 511/171  
 541/181  
 GGT CAG TTG TAT GGC CAT GTC GGC GAT GAA CTG GCG ACG CCG TCT TCC GCA ATT CTT GAT ATT GAA CGC AAA GTT ACG GCA TTG ACC CGC  
 G Q L Y G H V A D E L A T P S S A I L D I E R K V T A L T R  
 601/201  
 631/211  
 AGC GGC GAG CTG CCG GTT GAT AAC TTT GGC GTA CCG CTG GCG GGA AGC CTG ATC CCC TGG ATC GAC AAA CAG CTC GAT AAC GGC CAG AGC  
 S G E L P V D N F G V P L A G S L I P W I D K Q L D N G Q S  
 691/231  
 721/241  
 CGC GAA GAG TGG AAA GGC CAG GCG GAA ACC AAC AAG ATT CTC AAT ACT GCC TCT GTG ATT CCG GTT GAT GGT TTG TGT GTG CGC GTC GGC  
 R E E W K G Q A E T N K I L N T A S V I P V D G L C V R V G  
 781/261  
 811/271  
 GCG CTG CGC TGT CAC AGC CAG GCG TTC ACC ATC AAG CTG AAA GAG GTA TCC ATT CCG ACG GTG GAA GAA CTG CTG GCG GCA CAT AAT  
 A L R C H S Q A F T I K L K K E V S I P T V E E L L A A H N  
 871/291  
 901/301  
 CCG TGG GCG AAA GTG GTG CCG AAC GAT CGT GAT ATC ACT ATG CGC GAA TTA ACC CCG GCG GTG ACC GGC ACG TTG ACT ACG CCG GTT  
 P W A K V V P N D R D I T M R E L T P A A V T G T L T T P V  
 961/321  
 991/331  
 GGT CGT CTG AAG CTG AAC ATG GCG CCA GAG TTC TTG TCG GCG TTT ACC GTA GGC GAC CAG TTG TTA TGG GGC GCC GCC GAG CCG CTG  
 G R L R K L N M G P E F L S A F T V G D Q L L W G A A E P L  
 1051/351  
 1081/361  
 CGT CGA ATG CTG CGC CAG TTG GCG TAG  
 R R M L R Q L A \*

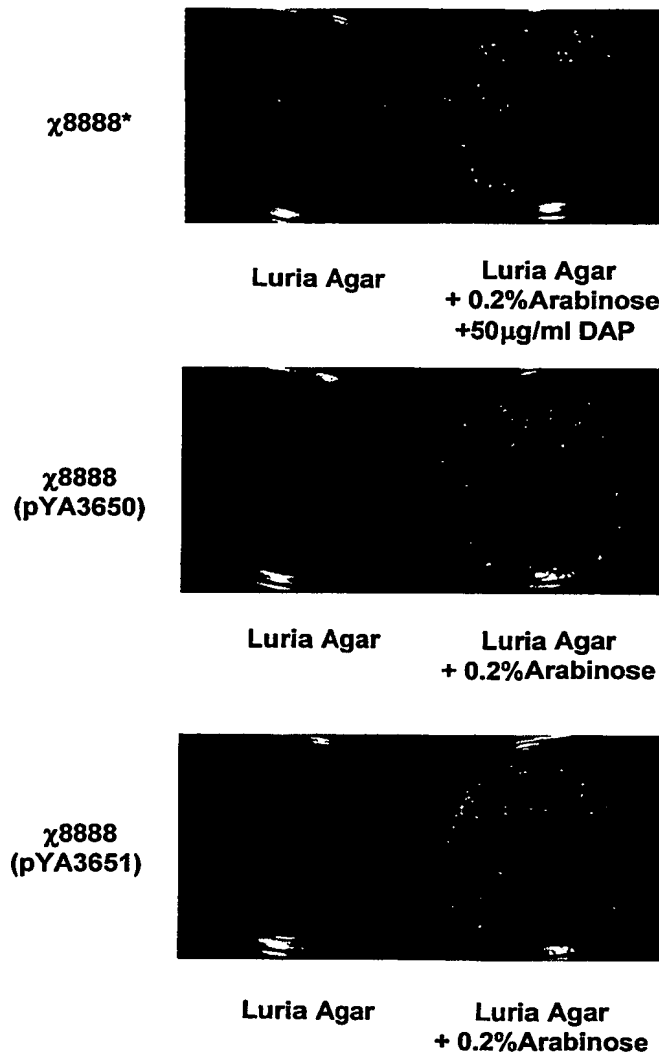
**FIGURE 45. Immuno Blot Analysis on AraC P<sub>BAD</sub> Asd Vectors Using Rabbit Anti-Asd Serum.**



**Lanes:**

1. χ6212 (*E. coli* Δ*asdA4*)
2. χ8276 (*S. typhimurium* UK-1 Δ*asdA16*)
3. pYA3530 Asd-GTG (Fig. 2A) in χ8276
4. pYA3450 Asd-ATG in χ8276
5. pYA3565 Asd-TTG in χ8276
6. pYA3450 Asd-ATG in χ6212
7. pYA3530 Asd-GTG in χ6212
8. pYA3656 Asd-TTG in χ6212

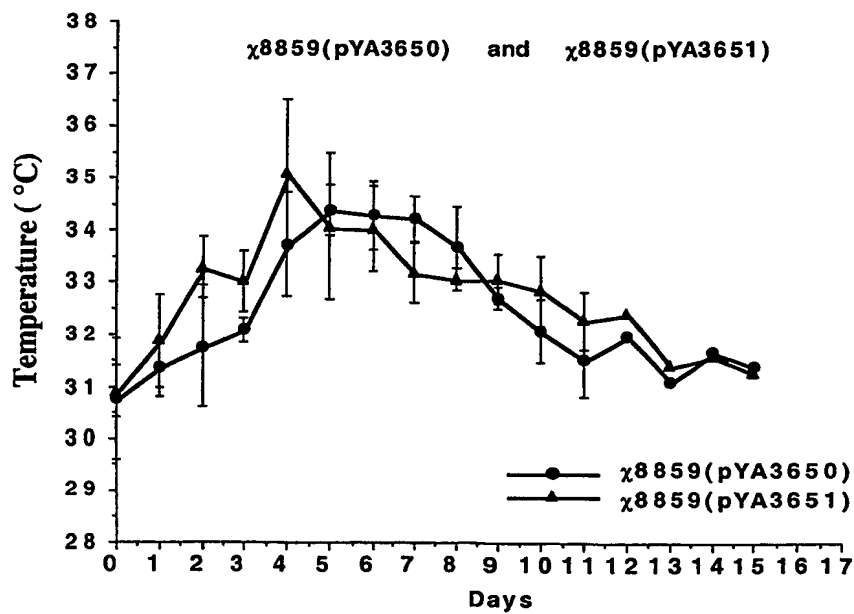
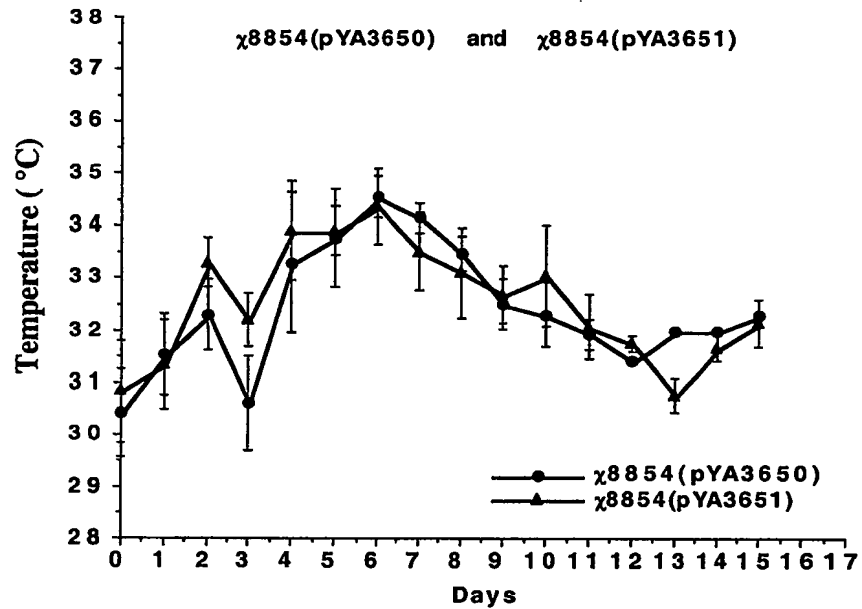
**FIGURE 46. DAP-less and muramic-less death in host strain with DNA vaccine vectors**



The results shows phenotypic properties of recombinant host-vector strains displaying arabinose-dependent growth and regulated cell lysis in the absence of arabinose.

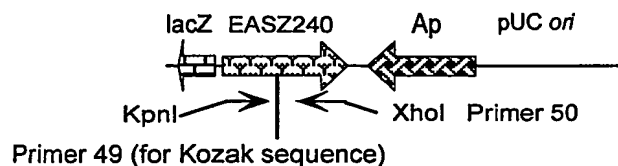
$\chi$ 8888  $\Delta asdA::araC$   $P_{BAD}$   $c2$ ,  $\Delta P_{murA}::araC$   $P_{BAD}$   $murA$ ,  $\Delta araBAD$ ,  $\Delta araE$ ,  $\Delta endA$ ,  $\Delta gmd-fcl$ ,  $\Delta relA$

**FIGURE 47. Change in body temperature as a consequence of oral immunization of 8-week-old female BALB/c mice with live host-vector systems for delivery of DNA vaccine vectors by regulatable cell lysis in vivo**

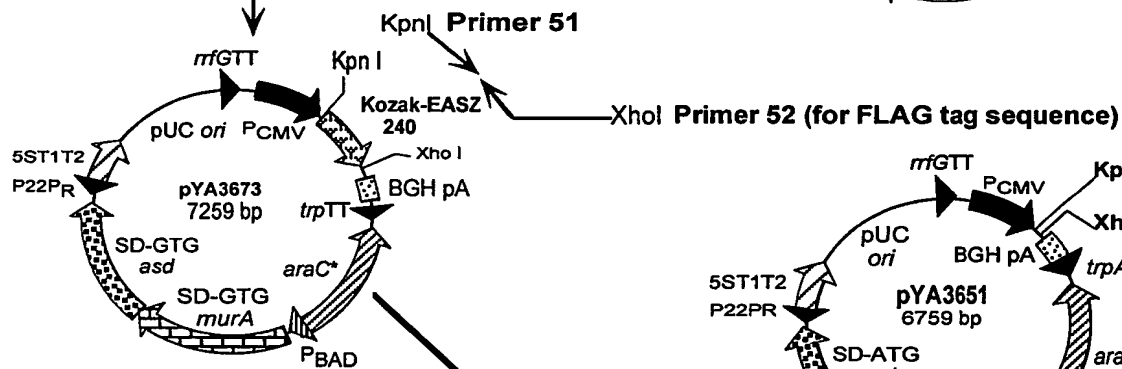


**FIGURE 48. Construction of pYA3674 (pYA3650 specifying expression *Eimeria acervulina* EASZ240- FLAG) and pYA3675 (pYA3651 specifying EASZ240-FLAG)**

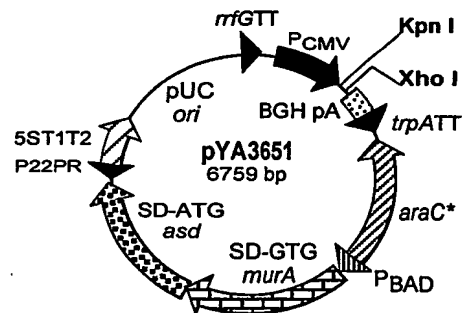
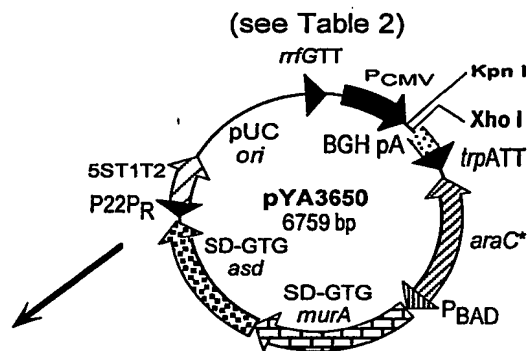
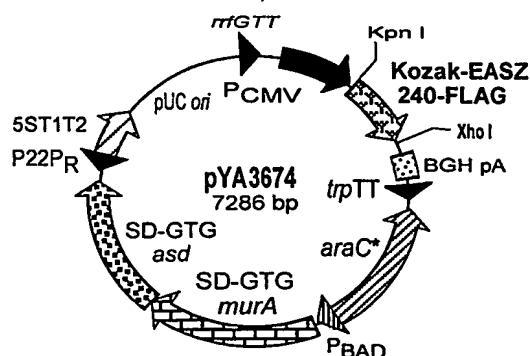
**pUC19-EASZ240(#1)**



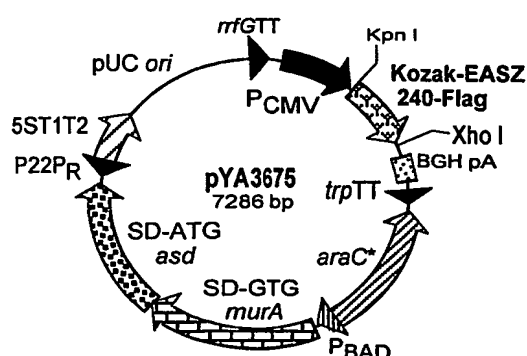
Introduce Kozak sequence and initiation codon (ATG) into EASZ240 by PCR using primer 49 and 50, digest 579 bp PCR product with KpnI and XhoI and insert into KpnI and XhoI of pYA3650



Introduce FLAG tag into c-terminal of EASZ 240 by PCR using Primer 51 and 52, digest 611 bp PCR product with KpnI and XhoI and insert at KpnI and XhoI of pYA3650

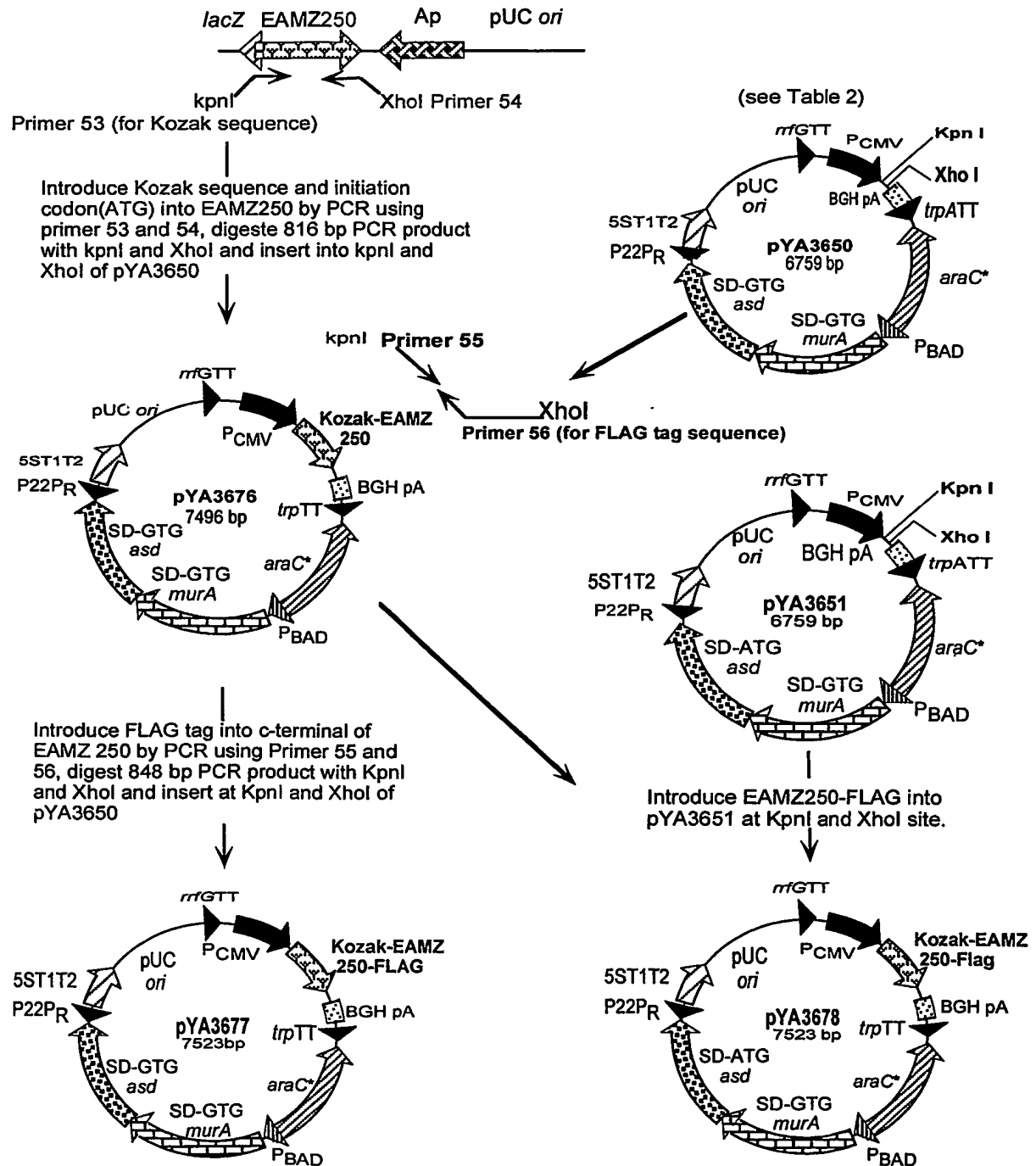


Introduce EASZ240-FLAG into pYA3651 at KpnI and XhoI site.



**FIGURE 49. Construction of pYA3677 (pYA3650 specifying the expression of the *Eimeria acervulina* EAMZ250-FLAG) and pYA3678 (pYA3651 specifying EAMZ250-FLAG)**

**pUC19-EAMZ250 (#3)**



**FIGURE 50. DNA and amino acid sequences of EASZ-240 with FLAG fusion  
 in pYA3674 and pYA3675**

5' - G GTA CCA GGA GCC GCC ACC ATG GCA-  
 KpnI SD Kozak start A  
 codon

cgt ttc ttt gta ttt cct tac tca gtt aaa atg ggt gaa gag gct gat act cag gcg tgg gat acc tca gtg aag gaa tgg ctc gtg gat  
 R F V F P Y S V K M G E A D T Q A W D T S V K E W L V D  
 1th a.a of EASZ240 →

acg ggg aag gta tac gcc gcc ggc att gct agc att gca gat ggg tgc cgc ctg ttt ggc gct gca ata gac aat ggg gag gat gcg tgg  
 T G K V Y A G I A S I A D G C R L F G A A I D N G E D A W

agt cag ttg gtg aag aca gga tat cag att gaa gtg ctt caa gag gac ggc tct tca act caa gag gac tgc gat gaa gcg gaa acc ctg  
 S Q L V K T G Y Q I E V L Q E D G S S T Q E D C D E A E T L

cgg caa gca att gtt gac gcc cgt gcc cca aac ggt gtt tat att gga gga att aaa tat aaa ctc gca gaa gtt aaa cgt gat ttc acc  
 R Q A I V D G R A P N G V Y I G G I K Y K L A E V K R D F T

tat aac gac cag aac tac gac gtg gcg att ttg ggg aag aac aag ggt ggc ggt ttc ctg att aag act ccg aac gac aat gtg gtg att  
 Y N D Q N Y D V A I L G K N K G G F L I K T P N D N V I

gct ctt tat gac gag gag aaa gag cag aac aaa gca gat gcg ctg aca acg gca ctt gcc ttc gct gag tac ctg tac cag ggc ggc ttc  
 A L Y D E E K E Q N K A D A L T T A L A F A E Y L Y Q G F  
 last a.a of EASZ240 →

GAT TAT AAA GAT GAT GAT AAA TAA TAA CTC GAG-3'  
 D Y K D D D D K \* \* XhoI  
 FLAG tag stop codon

**FIGURE 51. DNA and amino acid sequences of EAMZ-250 with FLAG fusion in pYA3677 and pYA3678**

5' - G GTA CCA GGA GCC GCC ACC ATG GCT-  
 KpnI SD Kozak start A  
 codon

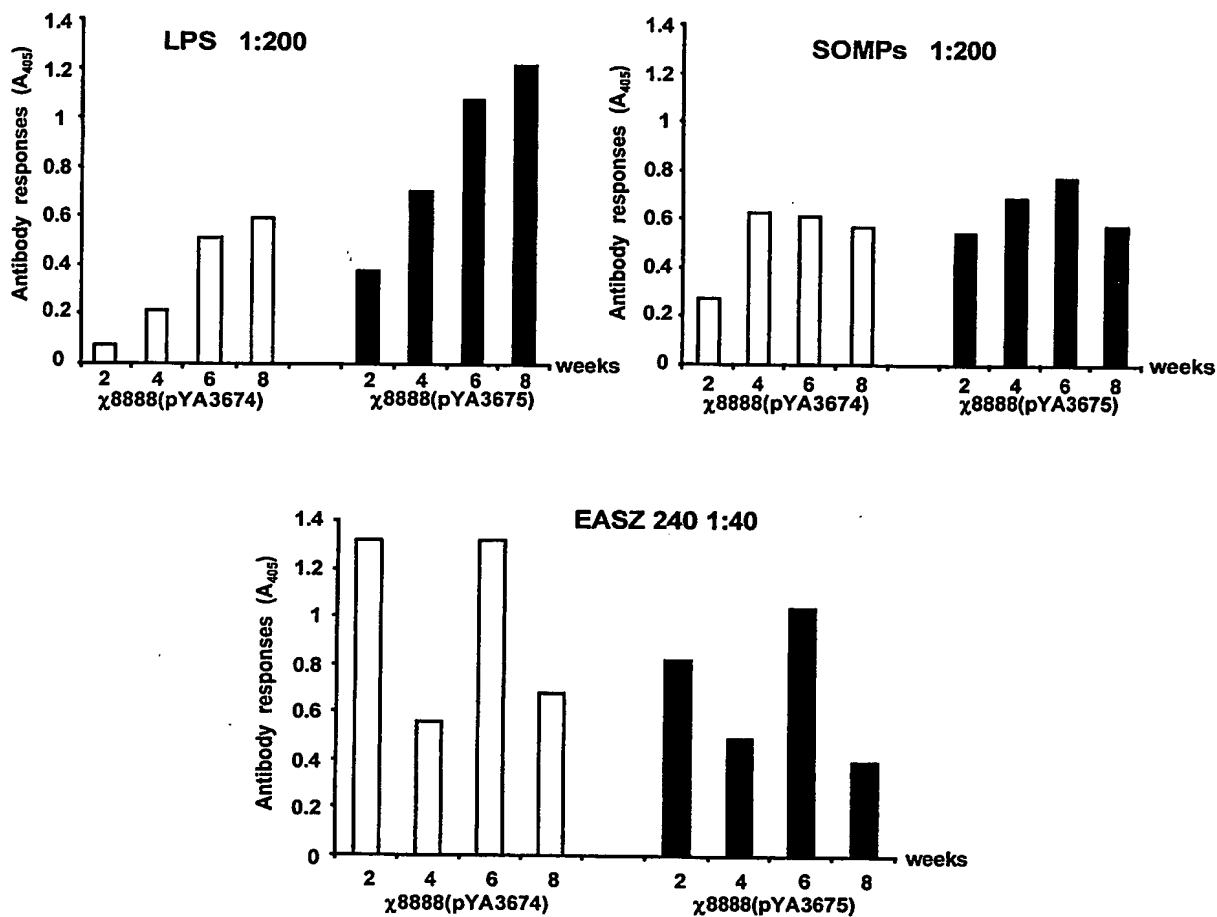
cct ttg ccc ttt tct cct cct tct aca ccg gtc tct cct cct tct aca ccg gtc tct cct cct tct aca  
 P L P F S P P S T P V S P P S T P V S T P S P P S T  
 1th a.a of EAMZ250 →

cca gtc tct cct cct aca ccg gtt tgc cct cct tct aca ccg gtc tgc cct cct tct aca ccg gtc  
 P V S P P S T P V S P P S T P V S T P V S P P S T P V  
 tgc cct cct tct aca ccg gtc tgc cct cct tct aca ccg gtc tgc cct cct tct aca ccg gtt tca cca  
 S P P S T P V S P P S T P V S T P V S P P S T P V S P  
 cct tct aca ccg gtc tca cca cct tct aca ccg gtt tgc cct cct tcc tct cgc cct ggt ggc gtt ggc gtc aat tca agt ctg  
 P S T P V S P P S T P V S P P S P A P G A V G V N S S L  
 tgc caa cgc tca acc tcc gag cac tgg cac gcg tca gtt tcc gtg cag ttt gag cgc tgg cga gat cgc acg cct gcc tct ggc cta cga  
 S Q R S T S E H W H A S V S V Q F E R W R D R T P A S G L R  
 ttc gct cca ctc gcc gaa ggg tgg gca att ctg act gcc gca agt tgt aac ctc cac aac atc agg cag cgc cca ggc tcc tct gct gca  
 F A P L A E G W A I L T A A S C N L H N I R Q R P G S A A  
 gac cgt cgg cat tgc act cgc tcc act cgc tcc agt cgc cgc atg tgc aga cgt cat cgt cac aag ggc gga ctt cgt ggg ttt gtt tca  
 D R R H C T R S T R S S R R M S R R H R H K G L R G F V S  
 cga tgc cgt cgg agc gga tgc tgc agg ttc tct tca ttt gct tct cgc acg att cgc tcc aag ctt aca ggt tac ggt gtc gct gac gtc  
 R C R R S G C C R F S S F A S P T I R S K L T G Y G V A D V  
 ggc tgt gga gta ctc ttc gta ctc cgt cac acc gca cgc cgt atc ctc gcg cgt tgc GAT TAT AAA GAT GAT GAT AAA TAA TAA  
 G C G V L F V L R H T A R R I L A R S D Y K D D D K \* \*  
 last a.a of EAMZ250 FLAG tag Stop codon

CTC GAG-3'  
 XhoI



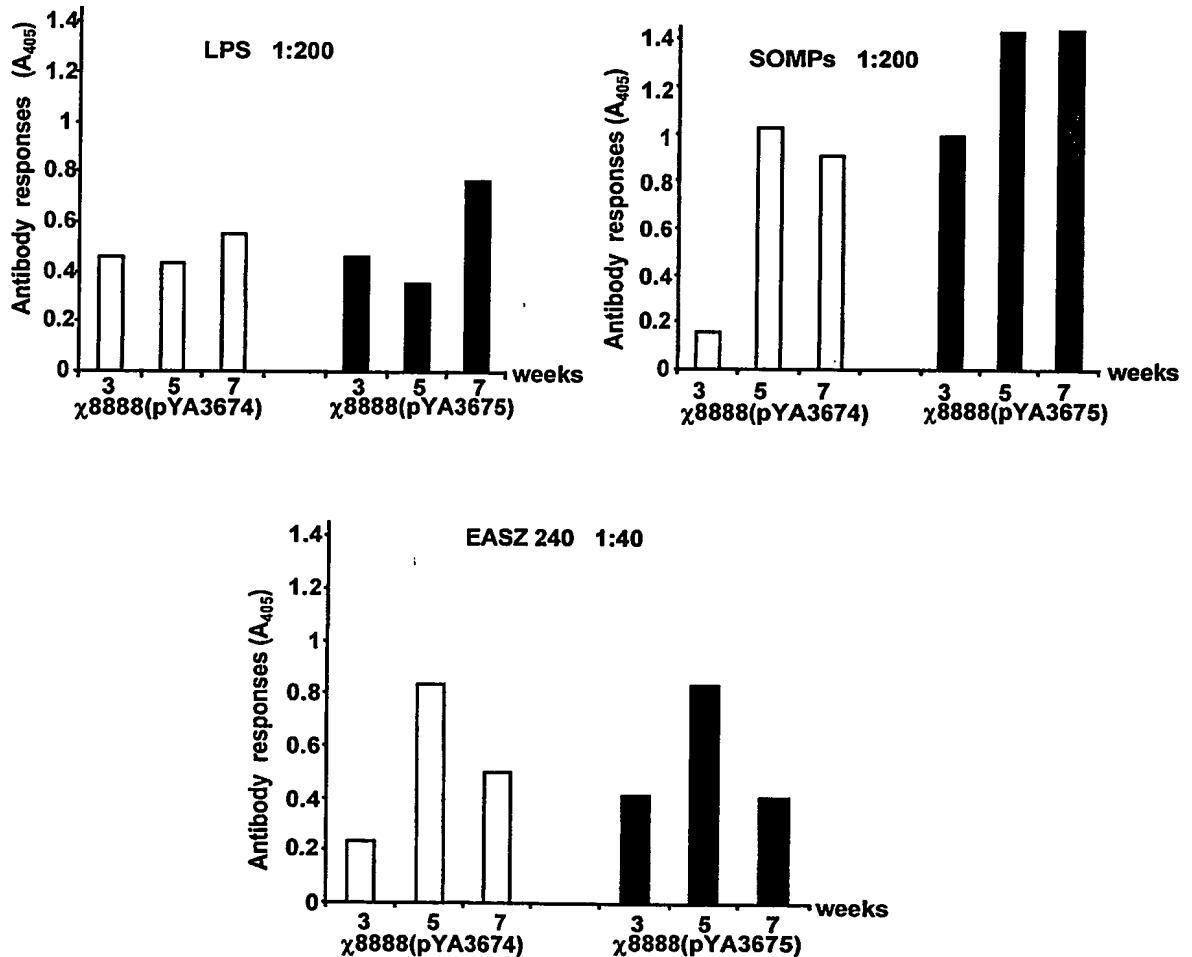
**FIGURE 52. Serum of mice IgG responses to *S. typhimurium* LPS, SOMPs and to EASZ 240.**



$\chi 8888(pYA3674)$  with GUG translation start codon of *asd* mRNA induced more rapid cell wall-less death to elicit less of an IgG responses to LPS and SOMPs but a higher response to EASZ240.

Immunity to *S. typhimurium* is a plus but is not the objective and a very strong induction of immunity to *Salmonella* antigens could compete in induction of desired immune responses to specified protective antigen.

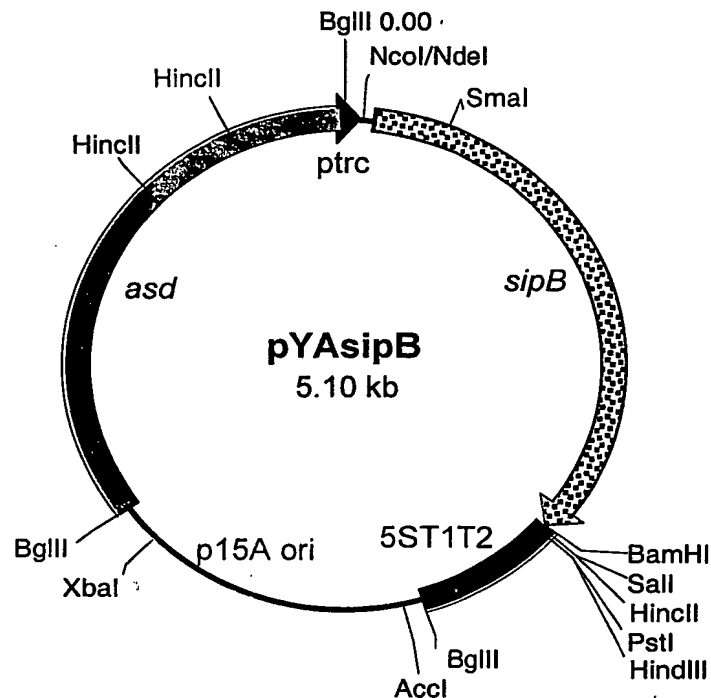
**FIGURE 53. Chicken Serum IgG responses to *S. typhimurium* LPS, SOMPs and to EASZ 240**



The data represent IgG antibody levels induced in chickens orally immunized with  $\chi$ 8888 (pYA3674) and  $\chi$ 8888 (pYA3675) at the indicated weeks after immunization.

Both strains elicited anti-LPS, anti-SOMPs and anti-EASZ 240 IgG.

**FIGURE 54. Cloning of *sipB* in Asd vector pYA3332**



1818 bp of *sipB* gene (*sipB*-8 to *sipB*+18) was PCR amplified from the *S. typhimurium* UK-1 chromosomal DNA with Primer *sipB*-NdeI and *sipB*-BamHI. This fragment was digested with NdeI enzyme and then filled-in with Klenow enzyme and cut the other end with BamHI. Asd vector pYA3332 was digested with NcoI enzyme and then the overhang removed with a mung bean nuclease and then cut the other end with BamHI enzyme. Then ligate the *sipB* insert with the pYA3332 (blunt ligation).

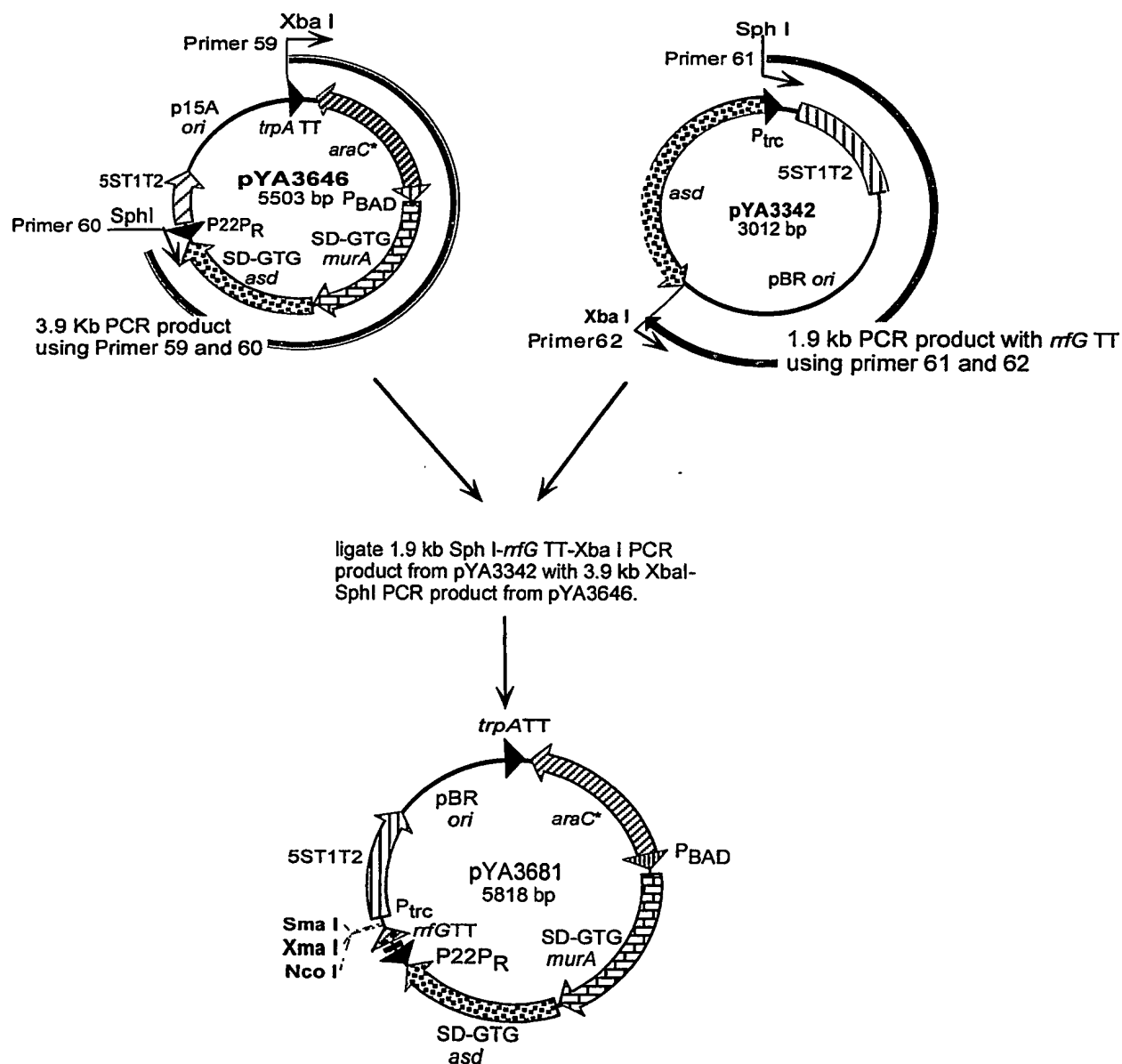
fused area sequence:

<u>AGGA</u>	AACAGACT	<u>ATG</u>	ACG	CAA
SD		M	T	Q
		<i>sipB</i>		

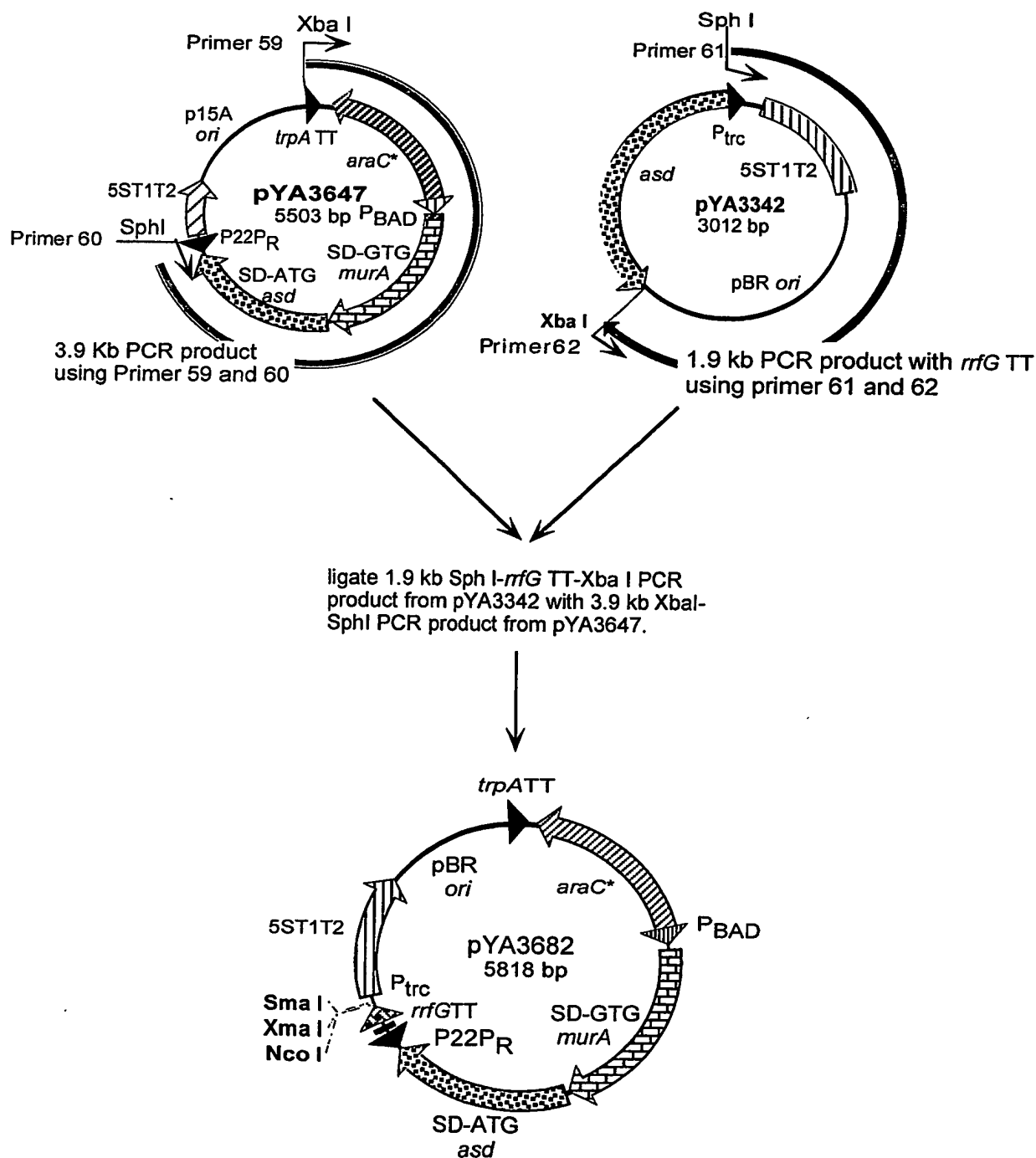
Primer 57. *sipB*-NdeI: 5'GCAATTCCATATGGTAAATGACGCAAGTAGCATTAG 3'

Primer 58. *sipB*-BamHI: 5'CCGATCCTTTATTTTGGCAGTTTTTATGCG 3'

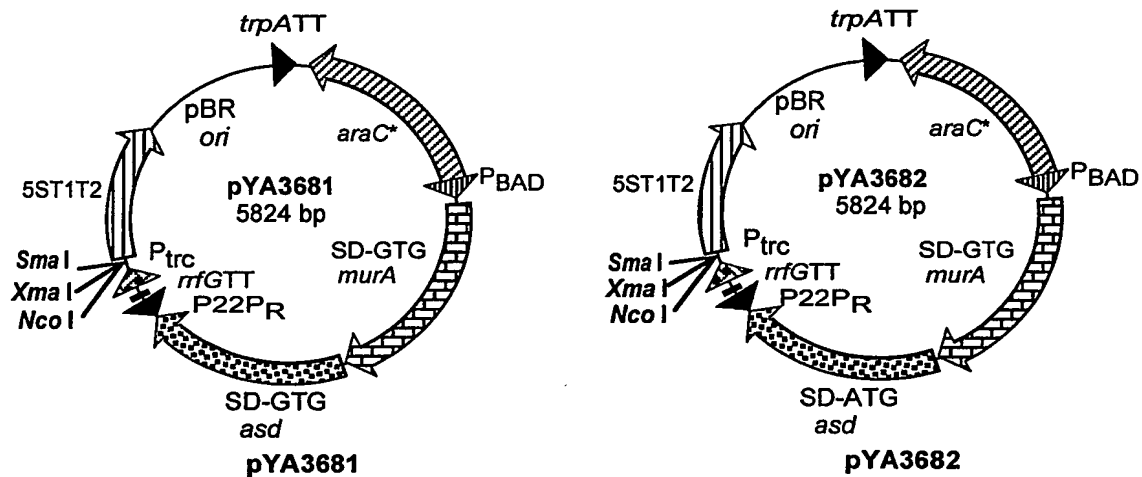
**FIGURE 55. Construction of pYA3681 (pYA3646 with the  $P_{trc}$ -MCS TT-pBR *ori* cassette)**



**Figure 56. Construction of pYA3682 (pYA3647 with the  $P_{trc}$ -MCS TT-pBR *ori* cassette)**



**FIGURES 55 and 56. Regulated lysis vector pYA3681 and pYA3682**



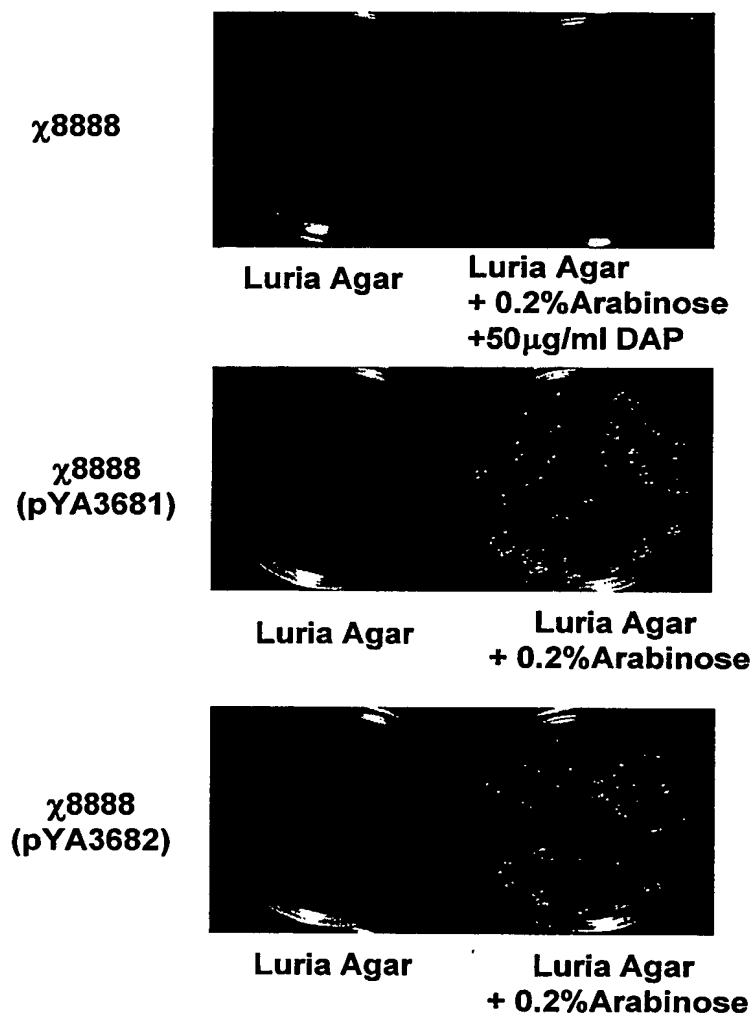
**pYA3681 and pYA3682 possess:**

- pBR *ori*.
- P<sub>trc</sub> promoter for expressing antigen gene.
- araCP*<sub>BAD</sub> activator-promoter.
- two genes for essential enzymes for synthesis of rigid layer of the bacterial cell wall, *asd* and *murA*.
- GTG start codon for *murA* and either GTG or ATG start codon for *asd* gene.
- P22P<sub>R</sub> promoter for synthesis of anti-sense mRNA of *asd* and *murA* genes.
- multiple transcription terminators to block transcription of genes in adjacent segments of vector.

**P<sub>trc</sub> promoter region sequence:**

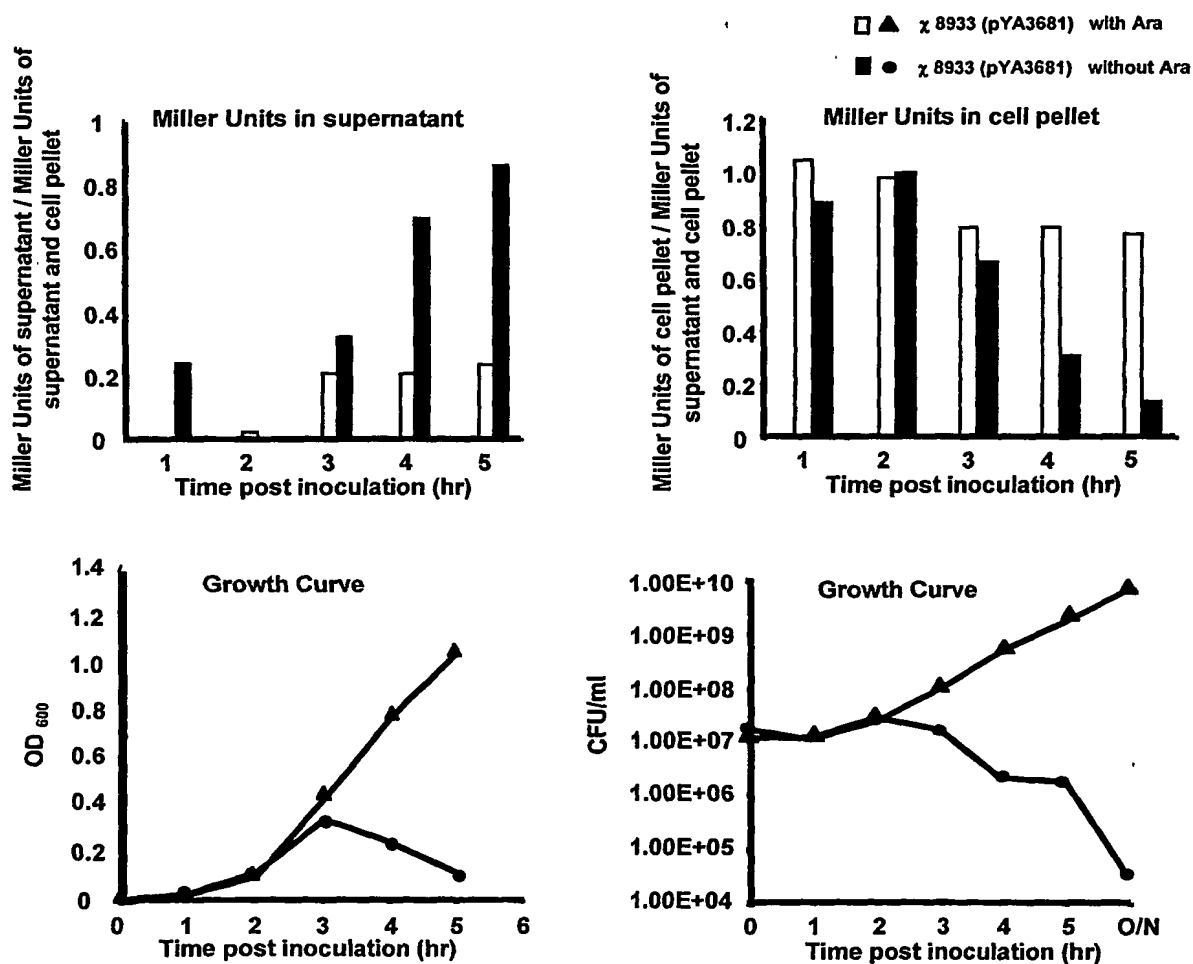
**P<sub>trc</sub>** →                      -35                      -10  
ATTCTGAAATGAGCTGTTGACAATTAATCATCCGGCTCGTATAATGTGT  
**SD**                      NcoI  
GGAATTGTGAGCGGATAACAATTTACACAGGAACAGACCATGGGAA  
**SmaI/XmaI**  
TTCGCAATTCCCGGGGATCCGTCGACCTGCAGCCAAGCTCCCAAGCTT

**FIGURE 57. DAP-less and Muramic-less Death in host strain with lysis system vectors**



The result shows phenotypic properties of recombinant host-vector strains displaying arabinose-dependent growth and regulated cell lysis in the absence of arabinose.

$\chi 8888$ :  $\Delta asdA::araC P_{BAD} c2, \Delta P_{murA}::araC P_{BAD} murA, \Delta araBAD, \Delta araE, \Delta endA, \Delta gmd-fcl$  and  $\Delta relA$

**FIGURE 58.  $\beta$ -Galactosidase release by cell lysis**

$\beta$ -Galactosidase production from  $\chi$ 8933 (the *atrB13::MudJ* allele in  $\chi$ 8888) was used as a cytoplasmic protein marker and as an indicator of cell lysis in the examination of regulated bacterial lysis in vitro.

The ratio of  $\beta$ -Galactosidase activity in supernatant and cell-pellet revealed the extent of cell lysis.

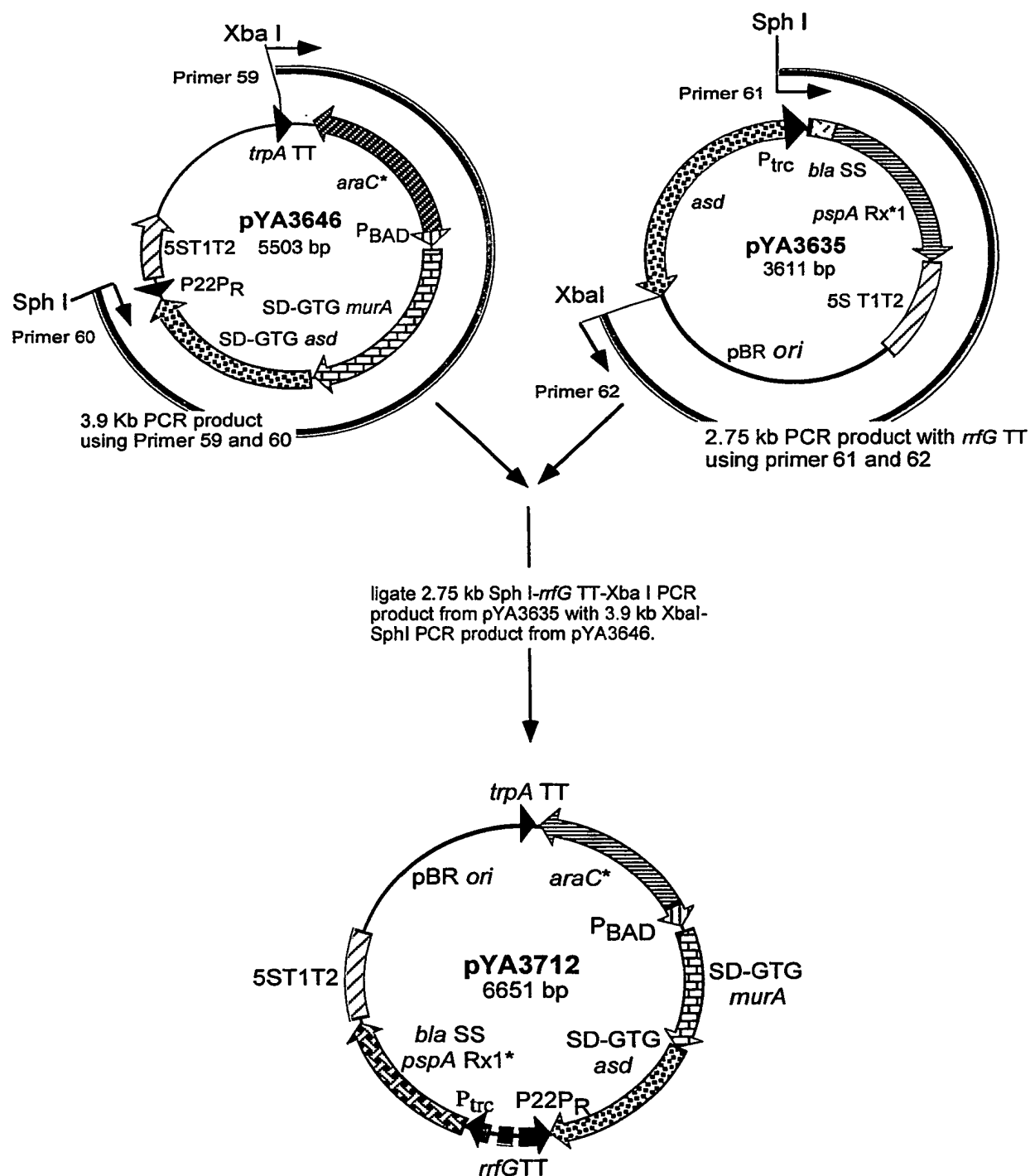
Inoculate (1:400) overnight culture (LB broth with 0.002% Ara) to fresh prewarm LB broth with 0.02% Ara,  $\beta$ -Galactosidase activity in supernatant and cell-pellet were assayed at indicated time point, respectively.

At 3 hr post inoculation (5.4 generations),  $\chi$ 8933 (pYA3681) growing in LB broth without Ara shows significant cell lysis and  $\beta$ -Galactosidase activity of supernatant.

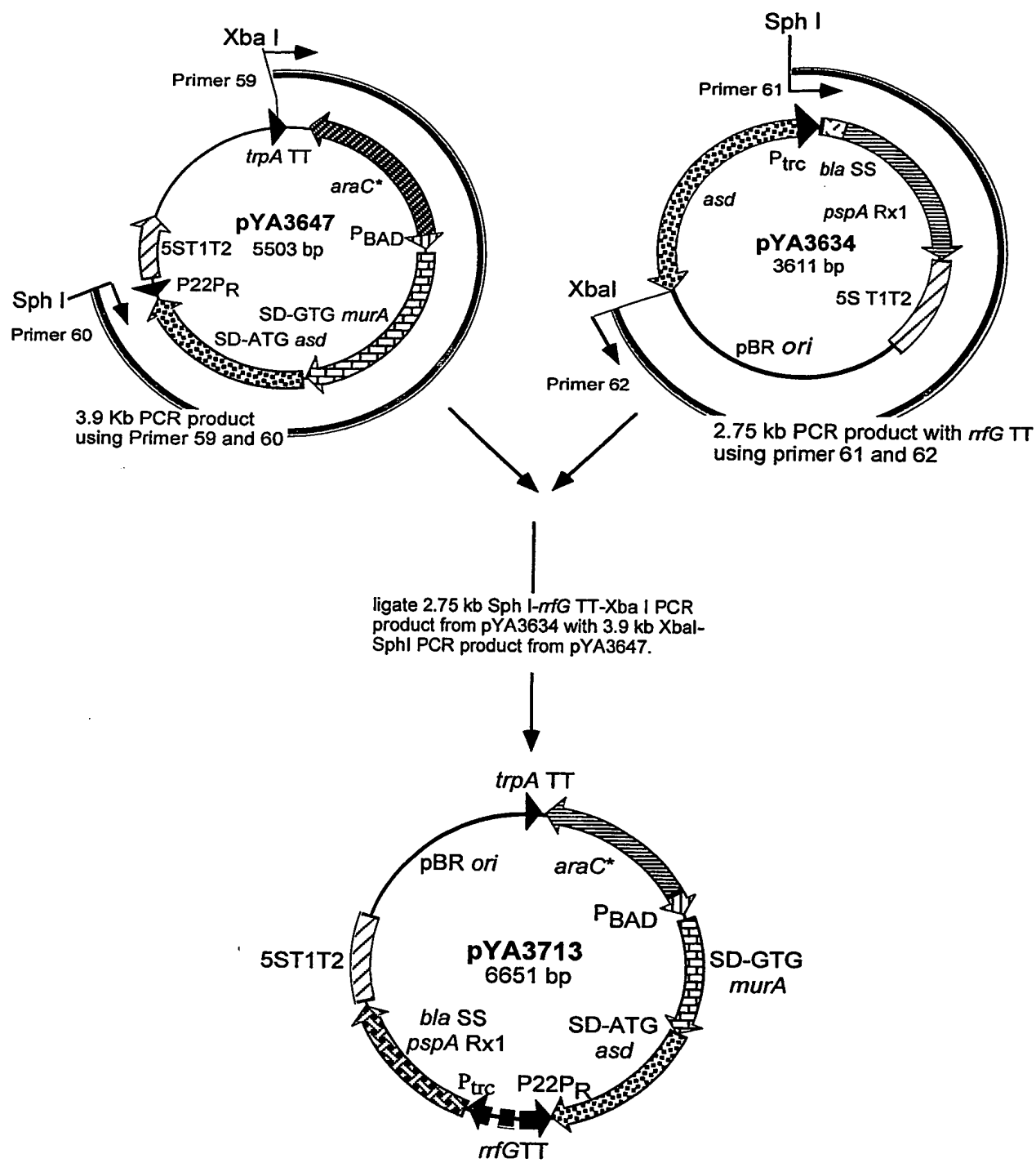
$\chi$ 8933- *atrB13::MudJ* allele in  $\chi$ 8888



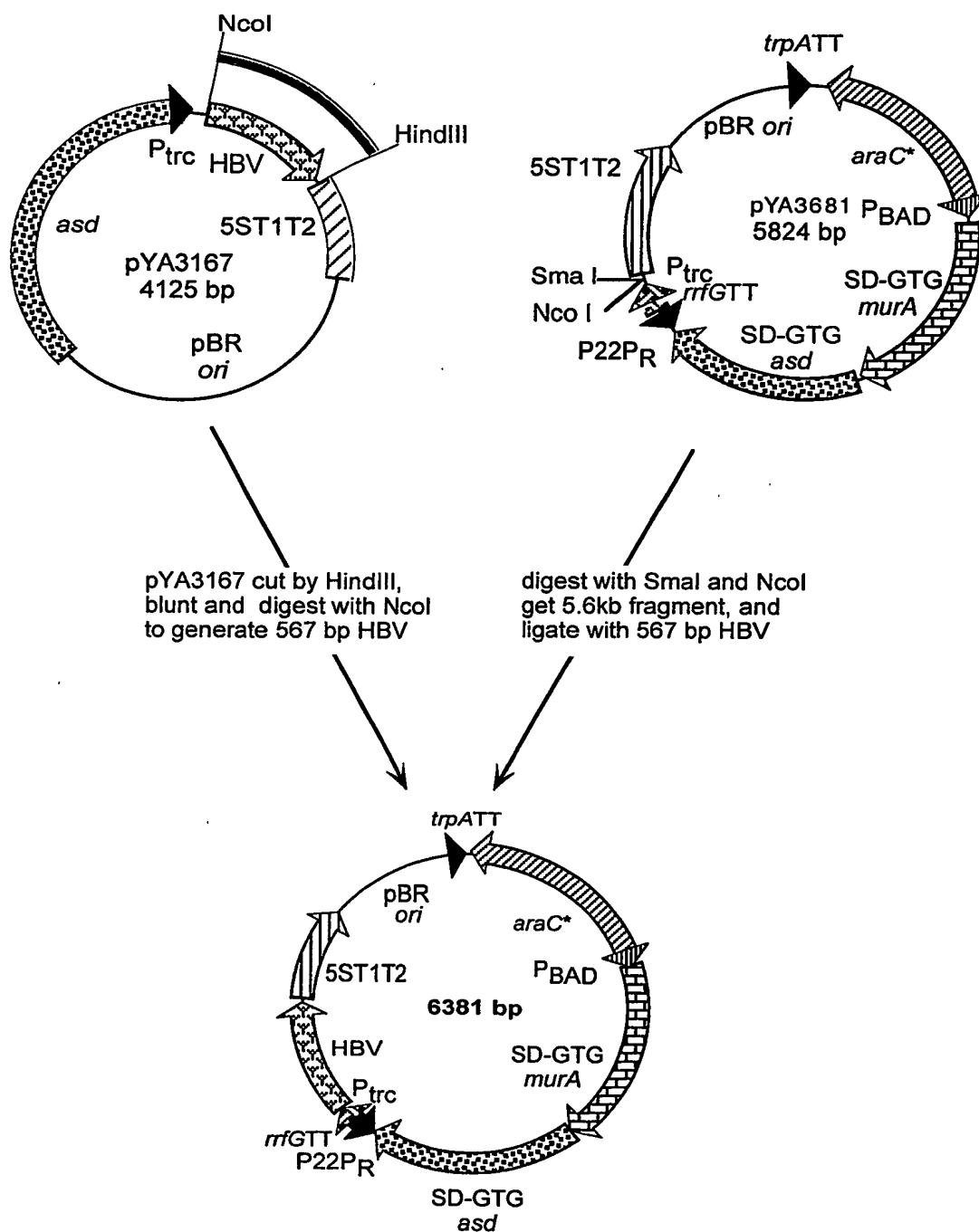
**FIGURE 59. Construction of pYA3712 (pYA3681 specifying the expression of codon-optimized rPspA-RX\*1)**



**FIGURE 60. Construction of pYA3713 (pYA3682 specifying the expression of rPspA-RX1)**



**FIGURE 61. Construction of the pYA3681 expressing the HBV core PreS1,S2 sequences.**



**FIGURE 62. DNA and amino acid sequences of HBV core gene with preS1 and pre S2 epitopes in pYA3681**

**Pttrc promoter**

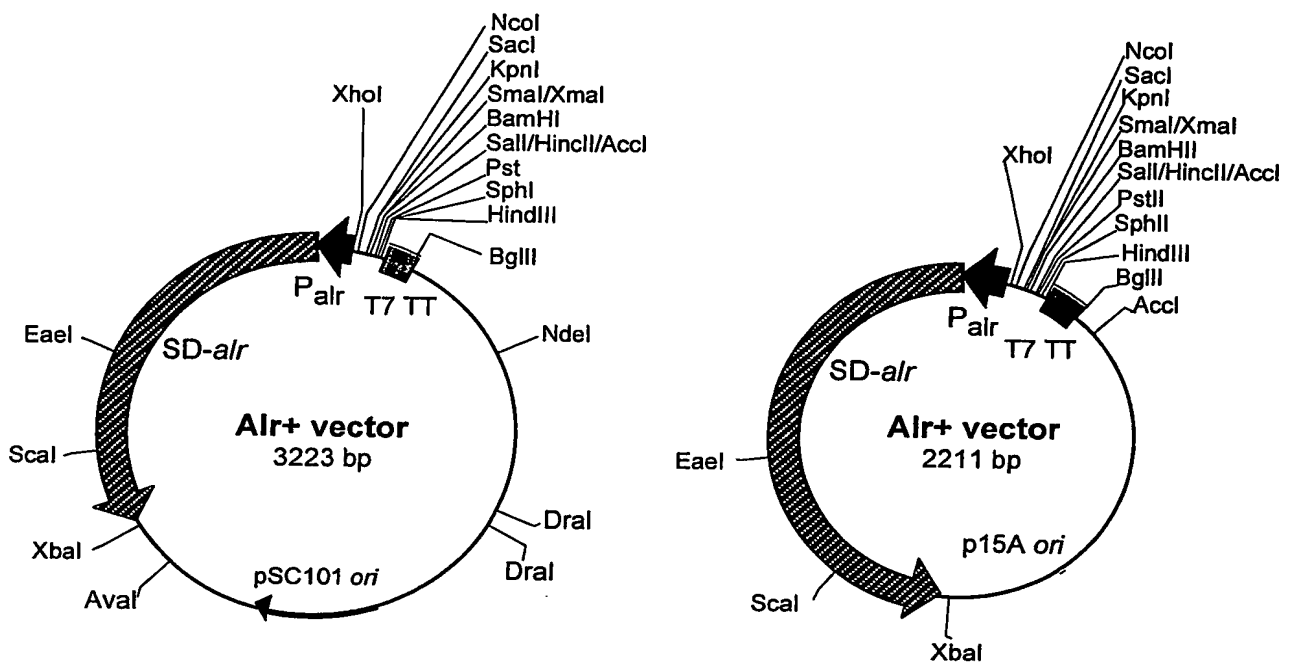
-35 -10  
TTGACAATTA ATCATCCGGC TCGTATAATG TGTGGAATTG TGAGCCGATA ACAATTTTCAC

SD  
ACAGGAAACA GACC

1/1	31/11
<u>ATG</u> GAC ATC GAC CCT TAT AAA GAA TTT GGA	GCT ACT GTG GAG TTA CTC TCG TTT TTG CCT
M D I D P Y K E F G	A T V E L L S F L P
61/21	91/31
TCT GAC TTC TTT CCT TCA GTA CGA GAT CTT	CTA GAT ACC GCC TCA GCT CTG TAT CGG GAA
S D F F P S V R D L	L D T A S A L Y R E
121/41	151/51
GCC TTA GAG TCT CCT GAG CAT TGT TCA CCT	CAC CAT ACT GCA CTC AGG CAA GCA ATT CTT
A L E S P E H C S P	H H T A L R Q A I L
181/61	211/71
TGC TGG GGG GAA CTA ATG ACT CTA GCT ACC	TGG GTG GGT GTT AAT ACT GCA AAT CCA GAT
C W G E L M T L A T	W V G V N T A N P D
241/81	271/91
TGG GAC TTC AAT CCC AAC AAG GAC ACC TGG	CCA GAC GCC AAC AAG GTA GGA GCT GGA GCA
W D F N P N K D T W	P D A N K V G A G A
301/101	331/111
TTC GGG TCT AGA GAC CTA GTA GTC AGT TAT	GTC AAC ACT AAT ATG GGC CTA AAG TTC AGG
F G S R D L V V S Y	V N T N M G L K F R
361/121	391/131
CAA CTC TTG TGG TTT CAC ATT TCT TGT CTC	ACT TTT GGA AGA GAA ACA GTT ATA GAG TAT
Q L L W F H I S C L	T F G R E T V I E Y
421/141	451/151
TTG GTG TCT TTC GGA GTG TGG ATT CGC ACT	CCT CCA GCT TAT AGA CCA CCA AAT GCC CCT
L V S F G V W I R T	P P A Y R P P N A P
481/161	511/171
ATC CTA TCA ACA CTT CCG GAG ACT ACT GTT	GTT AGA CGA CGA GGC AGG TCC CCT GGT GGC
I L S T L P E T T V	V R R R G R S P G G
541/181	
TCC AGT TCA GGA ACA GTA AAC CCT GTT	
S S S G T V N P V	

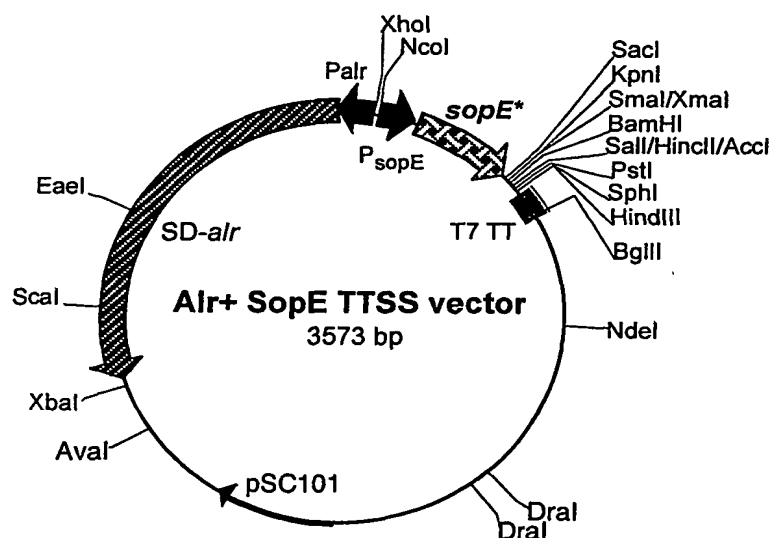
**HBcAg (1-75)-pre-S(27-53)-HBcAg(81-156)-pre-S2(133-143)**

**FIGURE 63. Construction of  $\text{Alr}^+$  plasmid vector with pSC101 *ori* and p15A *ori***



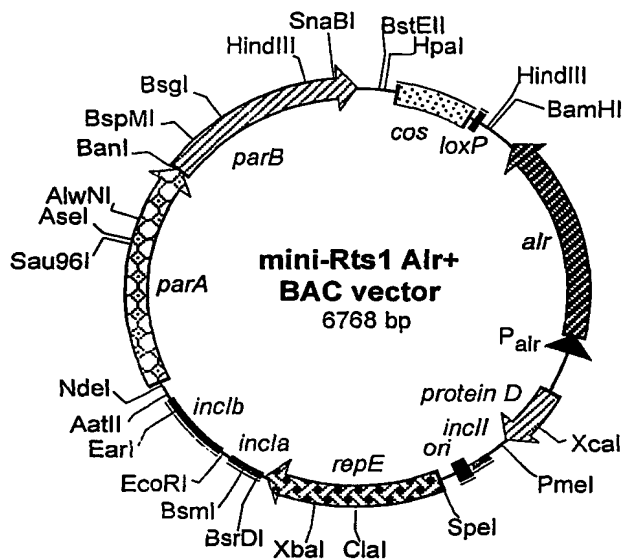
SD-*alr*: *alr*<sub>-10</sub> to *alr*<sub>1080</sub> + *T*  
*P*<sub>*alr*</sub>: *alr*<sub>-76</sub> to *alr*<sub>-11</sub>

**FIGURE 64. Construction of  $Alr^+$  plasmid vector with pSC101 *ori* to enable fusion of antigens with T-cell epitopes to the N-terminal end of the Type III effector SopE**



***sopE\**: 342 bp of PCR product (*sopE*-93 to +249) including 93bp *sopE* promoter region and 249 bp of *sopE* from *Salmonella***

**FIGURE 65. Construction of BAC vector with *IncIa*, *IncIb*, *IncII* genes and *Alr*<sup>+</sup> in place of antibiotic resistance genes**



*incII*, *ori*, *repE*, *incIa*, *incIb*, *parA* and *parB* sequences are from Rts1 plasmid